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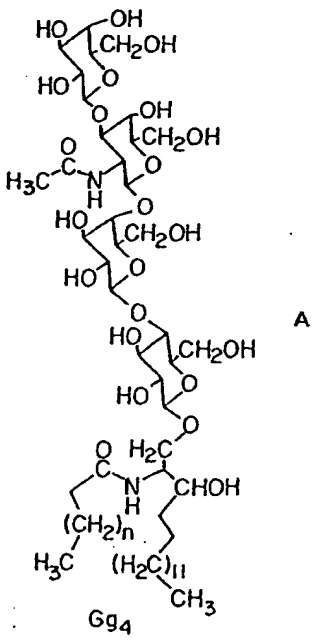
(57) Abstract

Methods for treating a glycolipid mediated state in a subject are described. An effective amount of at least one therapeutic compound represented by the structure A–B, in which A is a glycolipid receptor moiety and B is an active agent, is administered to a subject, such that treatment of the glycolipid mediated state occurs. Methods also include administering and effective amount of at least one therapeutic compound, or a pharmaceutically acceptable salt thereof, to a subject such that a disease state associated with a SLT is treated. Packaged pharmaceutical compositions for treating SLTs are described. The package includes a container for holding an effective amount of a pharmaceutical composition and instructions for using the pharmaceutical composition for treatment of SLT. The pharmaceutical composition includes at least one therapeutic compound for modulating a SLT in a subject.

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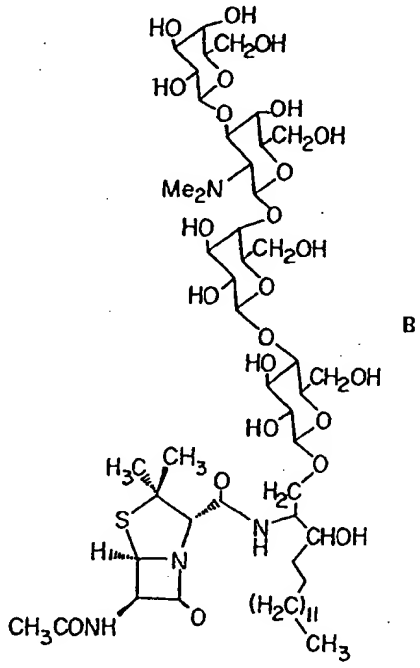


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# ANTIBIOTIC-LIGAND CONJUGATES AND METHODS OF USE THEREOF

## BACKGROUND

Glycolipids have been shown to be involved with the early steps of the infectious process associated with several pathogens. For example, it is believed that oligosaccharide moieties coupled to ceramide lipid bases are used by the infectious agents as anchors or adsoprtion moieties for invasion of the host cells. Many bacteria have been found to use extracellular membrane components, such as glycolipids to access host cells.

Shiga-like toxins (SLTs), a family of powerful, disease producing toxins, are produced by a common bacteria, *Escherichia coli*, found in humans and in animals. The term "SLT" is derived from the cytotoxic nature, structural and functional similarity to Shiga toxin which is a protein cytotoxin produced by *Shigella dysenteriae* type 1. This Shigella serotype is responsible for the most severe cases of bacillary dysentery. SLTs are also known as verotoxins (VTs) because many of the serotypes that produce this toxin were originally characterized as being vero cell toxinogenic. The first member of the family of SLTs to be isolated was cytotoxic for African Green Monkey (Vero) cells and was originally called verotoxin. Further, SLT producing *E. coli.* are a heterogeneous group of bacteria that belong to several different O:H:K serotypes; all having the ability to discharge one or more SLTs.

SLTs are multimeric proteins composed of an enzymatic (A) subunit and multiple (B) subunits responsible for toxin binding to receptors on host tissues. The binding B oligomers of the SLTs recognize host cell globoseries glycolipid receptors containing at a minimum, the disaccharide unit of αGal(1-4)βGal at the non-reducing terminus.

Foods of animal origin are a major source of human infection by SLTs. Infants, young children and the elderly are the most susceptible to SLT infection, however, anyone who eats contaminated food is prone to infection. Additionally, infection can be spread by person-to-person transmission which can be especially problematic in day care centers and nursing homes.

SLT-producing *E. coli* can also cause edema disease (ED) in swine. The often fatal disease occurs in weanling pigs, characterized by anorexia, edema of the eyelids and neurological abnormalities such as uncoordination and/or paralysis.

Antibiotics have been found to be contraindicated in the treatment of SLT producing *E. coli.* infection in humans and pigs. Antibiotics often enhance toxin production by the bacteria. Treatment of SLT infection generally relies on management of the physiological complications of the infection, e.g. fluid and electrolyte imbalances.

Although certain agents have been used to suppress infection of hosts by pathogens, there are limitations to their use. For example, the widespread use of antibiotics has increasingly led to the problem of resistant pathogens whose growth can no longer be inhibited by known antibiotics. Thus, the appearance of multi-drug resistant pathogens has prompted a search for new classes of compounds which are structurally and/or functionally different from existing drugs. Drugs having new mechanisms of action could be effective against resistant pathogens, where conventional drugs can no longer be used.

## SUMMARY OF THE INVENTION

This invention provides methods and compositions which are useful in the treatment of glycolipid mediated states, such as enteropathogenic and enterohemorrhagic *E. coli.* (EPEC and EHEC, respectively), e.g., verotoxin producing *E. coli.* (VTEC). Various pathogens, e.g., bacteria, invade host cells *via* attachment to or interaction with glycolipids which are associated with the host cell. The present invention serves to inhibit a pathogen from invading a host cell by providing a receptor molecule which has been modified with an active agent; the active agent in combination with the receptor molecule combine with the pathogen, thereby rendering it incapable of invading a host cell, or preferably, eradicating the pathogen.

The invention provides methods for treating a glycolipid mediated state in a subject by administering to the subject a therapeutically effective amount of a therapeutic compound, such that the glycolipid mediated state is treated. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

The present invention also provides methods of modulating interaction between a pathogenic microorganism and a glycolipid in a subject by administering to the subject a therapeutically effective amount of a therapeutic compound, such that interaction between a pathogenic microorganism and a glycolipid is modulated. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

The present invention provides methods for treating a state characterized by the presence of a shiga-like toxin in a subject by administering to a subject a therapeutically effective amount of a therapeutic compound, such that a state characterized by the presence of shiga-like toxin is treated. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

The present invention further provides compounds represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent. In one embodiment the glycolipid receptor moiety includes an oligosaccharide moiety coupled to a ceramide lipid base. In a preferred embodiment, the glycolipid receptor moiety is gangliotriaosyl ceramide galNAcβ1-4galβ1-4glc cer ($Gg_3$) or gangliotetraosyl ceramide galβ1-4galNAcβ1-4glc cer ($Gg_4$) and derivatives thereof. Active agents are coupled to the glycolipid receptor moiety and include antibiotics and carbocyclic compounds. Suitable antibiotics include penicillins, cephams, cephalosporins. Suitable carbocyclic compounds include adamantyl or acridine derivatives.

The present invention provides pharmaceutical compositions which include a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent and a pharmaceutically acceptable carrier. These pharmaceutical compositions are useful in treatment of glycolipid mediated states and for modulating interaction(s) between a pathogenic microorganism and a glycolipid in a subject.

The present invention also provides packaged therapeutic compositions for treating a glycolipid mediated state in a subject. The packaged therapeutic compositions include a container for holding a therapeutically effective amount of a therapeutic compound for treating a glycolipid mediated state in a subject and instructions for using the therapeutic composition for treating the glycolipid mediated state. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

The present invention further provides packaged therapeutic compositions for modulating interaction between a pathogenic microorganism and a glycolipid. The packaged therapeutic composition includes a container for holding a therapeutically effective amount of a therapeutic compound for modulating interaction between a pathogenic microorganism and a glycolipid in a subject and instructions for using the therapeutic composition for modulating interaction between the pathogenic microorganism and the glycolipid. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B depict deacylation of a ceramide and coupling of an antibiotic to the deacylated ceramide.

Figures 2A and 2B depict oxidation of the sphingosine double bond of glycolipids.

Figure 3 depicts coupling of an antibiotic with a deacylated ceramide.

Figure 4 depicts a ceramide functionalized with multiple antibiotics.

Figure 5 represents functionalization of LysoPE.

Figure 6 is a western blot showing conjugate binding with gp120.

Figure 7 shows glycolipid/lipid binding specificity.

Figure 8 shows enhanced inhibitory activity of $Gb_4$-ampicillan compared to ampicillin for uropathogenic *E. coli*.

Figure 9 shows inhibition of VT1 binding to $Gb_3$ phospholipid bilayer.

## DETAILED DESCRIPTION

The features and other details of the invention will now be more particularly described and pointed out in the claims. It will be understood that the particular embodiments of the invention are shown by way of illustration and not as limitations of the invention. The principle features of this invention can be employed in various embodiments without departing from the scope of the invention.

This invention pertains to methods and compositions which are useful in the treatment of glycolipid mediated states, such as enteropathogenic and enterohemorrhagic *E. coli.* (EPEC and EHEC, respectively), e.g., verotoxin producing *E. coli.* (VTEC).

The present invention pertains to methods for treating a glycolipid mediated state in a subject by administering to the subject a therapeutically effective amount of a therapeutic compound, such that the glycolipid mediated state is treated. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent. The methods of the invention can be used therapeutically to treat a subject afflicted by a pathogen or can be used prophylactically in a subject susceptible to pathogens. The methods of the invention are based, at least in

part, on inhibiting or preventing interaction between the cell membrane surface and the pathogen.

The language "treating a glycolipid mediated state" or "such that the glycolipid mediated state is treated" is intended to include changes in a glycolipid mediate state or condition, as described *infra*, such that physiological symptoms in a subject can be significantly diminished or minimized. The language also includes control, prevention or inhibition of physiological symptoms or effects associated with a disease state associated with glycolipid mediated states. In one preferred embodiment, the control of the glycolipid mediated state or condition is such that the glycolipid mediated state or condition is eradicated. In another preferred embodiment, the control is selective such that a particular targeted glycolipid mediated state, e.g., a pathogen, is controlled while other cells and physiological flora which are not detrimental to the subject are allowed to remain substantially uncontrolled or substantially unaffected, e.g., lymphocytes, red blood cells, white blood cells, platelets, growth factors, etc.

The term "pathogen" is art recognized and is intended to include disease producing agents, such as organisms capable of causing disease in a subject, e.g., a mammal, including, for example, bacteria, viruses, prions and fungi.

The term "glycolipid mediated state" is intended to include those disease states or conditions caused by or associated with one or more pathogens, e.g., bacteria. These glycolipid mediated states can include enterotoxins produced by pathogenic bacteria, e.g., *Escherichia coli*, and are known as shiga-like toxins (SLTs).

Without wishing to be bound by theory, host cell receptors for adhesion of pathogenic bacteria have often been found to comprise complex carbohydrates on the host cell surface. For the most part such carbohydrates have been found to be conjugated to lipid rather than protein, thus host/cell surface glycolipids play an important role as receptors for a variety of bacteria.

The major species recognized are glycolipids belonging to the ganglio series, globo series or sulfatide. Thus, many pathogenic bacteria have been shown to bind to the lipid-bound carbohydrate. The present invention pertains to ganglio series glycolipid recognition, since SLTs, such as verotoxin producing *E. coli* (VTEC) demonstrate a high binding affinity for these neutral glycosphingolipds and that this binding is distinct from that of enteropathogenic and commensal *E. coli* strains.

The term "SLT" is art recognized and is intended to include cytotoxins similar in structure and function to Shiga toxin. The term is also intended to include verotoxins, based upon structural similarity to shiga toxins by sequencing of relevant genes and are often referred to as SLT1. Known SLTs include SLT-1, SLTII, SLTIII. Variants of

SLTII (isolated and distinguished seriologically on the basis of gene sequence or host specificity) include SLTII; vtx2ha; SLTIIvh; vtx2hb; SLTIIc; SLTIIvp, etc. The term encompasses the presently unknown SLTs or variants thereof that may be discovered in the future, since their characterization as an SLT or variant thereof will be readily determinable by persons skilled in the art.

The term "subject" is intended to include mammals having a SLT, including one or more SLT related symptoms, or which are susceptible to pathogens producing SLTs. Examples of such subjects include humans, dogs, cats, pigs, cows, horses, rats and mice.

The language "therapeutically effective amount" of a therapeutic compound, described *infra*, is that amount of a therapeutic compound necessary or sufficient to perform its intended function within a subject, e.g., treat a glycolipid mediated state, or a state characterized by the presence of an SLT in a subject. An effective amount of the therapeutic compound can vary according to factors such as the amount of the causative agent already present in the subject, the age, sex, and weight of the subject, and the ability of the therapeutic compounds of the present invention to affect a state in the subject. One of ordinary skill in the art would be able to study the aforementioned factors and make a determination regarding the effective amount of the therapeutic compound without undue experimentation. An *in vitro* or *in vivo* assay also can be used to determine an "effective amount" of the therapeutic compounds described *infra*. The ordinarily skilled artisan would select an appropriate amount of the therapeutic compound for use in the aforementioned assay or as a therapeutic treatment.

A therapeutically effective amount preferably diminishes at least one symptom or effect associated with the glycolipid mediated state or SLT being treated by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. In a most preferred embodiment, the therapeutically effective amount diminishes at least one symptom or effect by at least about 90%, more preferably by at least about 95%, and still most preferably 100%. Assays can be designed by one skilled in the art to measure the diminishment of such symptoms and/or effects. Any art recognized assays capable of measuring such parameters are intended to be included as part of this invention. For example, if blood in the stool is treated, then the diminishment of blood in the stool can be measured before and after treatment using an art recognized technique. Likewise, if hypertension is the state being treated, then the pressure can be measured before and after treatment for measurement of diminishment of pressure using an art recognized technique.

The term "glycolipid receptor moiety" is intended to include those compounds which are glycolipids or are derived from glycolipids which are recognized by receptors on a cell surface, e.g., cell membrane or cell wall. The interaction between a glycolipid receptor moiety and the receptor can include adhesion, ionic interactions, charged interactions and the like. Typically glycolipid receptor moieties include an oligosaccharide moiety which is coupled to a ceramide lipid base. Preferred glycolipid receptor moieties are $Gg_3$-gangliotriaosyl ceramide, GalNAcβ1-4Galβ1-4 glucosyl ceramide, and $Gg_4$-gangliotetraosyl ceramide-Galβ1-3GalNAcβ1-4Galβ1-4 glucosyl ceramide (See also, U.S. Patent No. 5,521,282, the contents thereof are incorporated by reference.) In certain embodiments, the receptor moiety is not a $Gb_3$ or a $Gb_4$ moiety. In other embodiments, the receptor moiety is not those described in U.S. Patent 5,466,681.

For example, bacterial/host cell plasma membrane attachment is an important virulence trait for pathogenic bacteria. Many specific bacterial appendages, and adhesion molecules contained within such appendages, have been devised to maintain the close apposition of prokaryotic and eukaryotic cell surface membranes. Apart from the obvious benefit of preventing bacterial removal by nonspecific shear forces, such interactions can provide the basis for the development of specific biological niches for particular microorganisms. Such niches may involve the specific modification of the host cell plasma membrane to better accommodate the requirements of the bacteria. For enteropathogenic (EPEC) and enterohemorrhagic *E. coli* (EHEC, including VTEC), such parasitic interactions include modification of the host/cell plasma membrane architecture and submembrane reorganization due to complex signal transduction pathways between the attached organism and the host cytoskeletal network. This results in an 'attaching and effacing' (eae) lesion, in which local microvilli are lost, the host cell plasma membrane 'cups' the attached bacterium and actin is polymerized beneath the attachment site. Attachment *per se* can therefore cause significant changes in host cell physiology which of itself may induce pathology, e.g., in the case of gastrointestinal organisms, diarrhea.

Several bacterial products necessary for attachment have been identified, indicating the complexity of the virulence factor. Localized adhesion clusters characteristic of EPEC on the surface of epithelial cells are dependent on the presence of a 57 mDa plasmid which contains the gene encoding the structural subunit of the bundle-forming pilus. Expression of the *eaeA* gene product 'intimin', a 94 kDa outer membrane protein, is involved in, but not sufficient for, EPEC/host cell adhesion to form the eae lesion. A second chromosomal gene, *eaeB*, has been identified which is

also necessary for intimate host cell attachment. The expression of intimin is regulated by plasmid encoded factors which modulate virulence which further indicates the complexity of this system. Transfection of nonadherent *E. coli* with *eaeA* +/- *eaeB* does not result in the induction of epithelial cell adherence. *Eae* mutants still bind to host cells. Thus although *eae* is required for intimate host cell attachment, another factor may be required for initial host cell recognition and binding. A homologue of intimin has been identified in VTEC. The identification of the bundle forming pilus (bfp) in EPEC provides the mechanism for the initial host cell attachment of EPEC.

The host cell receptors for adhesins of pathogenic bacteria are believed to comprise complex carbohydrates on the host cell surface. For the most part such carbohydrates have been found to be conjugated to lipid rather than protein and play an important role as receptors for a variety of bacteria.

The common receptor activity of ganglio series glycolipids (primarily $Gg_3$-gangliotriaosyl ceramide, GalNAcβ1-4Galβ1-4 glucosyl ceramide and $Gg_4$-gangliotetraosyl ceramide-Galβ1-3GalNAcβ1-4Galβ1-4 glucosyl ceramide) for pathogenic bacteria was first demonstrated by studies by Krivan who noted that many respiratory pathogens bound to these glycolipids as demonstrated by TLC overlay *in vitro* (Krivan, H.C. *et al.* "Many Pulmonary Pathogenic Bacteria Bind Specifically to the Carbohydrate Sequence GalNAcβ1-4 Gal Found in Some Glycolipids" *PNAS* 85:6157-6161 (1988)). It has subsequently been established that *Helicobacter pylori* shares this binding specificity and an adhesin responsible for this binding has been purified and identified. Similarly, an adhesin responsible for related binding specificity for *Hemophilus influenza* has been isolated and purified.

Pathogens which bind to $Gg_3$ and or $Gg_4$ *in vitro* also bind to the phospholipid, phosphatidyl ethanolamine (PE). Further, binding studies to cells which contain or lack PE, suggest that PE is a significant receptor to mediate bacterial attachment to eukaryotic cells. Not wishing to be bound by theory, it is believed that the binding to eukaryotic cell surface PE allows bacteria to preferentially target apoptotic cells. The loss of plasma membrane phospholipid asymmetry is an early marker of programmed cell death. Thus PE, normally located, for the most part, on the inner leaflet of the plasma membrane bilayer becomes available at the outer leaflet for bacterial binding. Preferential binding of bacteria to apoptotic cells may allow for the more efficient acquisition of nutrients by the microorganism. Apoptosis has been shown to play a significant role in the turnover of both the respiratory and gastrointestinal epithelia and thus attachment of one bacterium may facilitate that of another.

The term "active agent" is intended to include those compounds which inhibit, eliminate, or prevent enterotoxins such as SLTs from affecting host cells of the subject. For example, the active agent can be an antibiotic known to those skilled in the art. The term "antibiotic" is art recognized and is intended to include those substances produced by growing microorganisms and synthetic derivatives thereof, which eliminate or inhibit growth of pathogens and are selectively toxic to the pathogen while producing minimal or no deleterious effects upon the infected host subject. Suitable examples of antibiotics include, but are not limited to, the principle classes of aminoglycosides, cephalosporins, chloramphenicols, fuscidic acids, macrolides, penicillins, polymixins, tetracyclines and streptomycins. Preferably, the active agents of the invention include penicillins, cephams, cephalosporins and carbocyclic compounds.

The term "carbocyclic compound" is intended to include carbon cage compounds, such as adamantanes as well as acridines and derivatives thereof. Moreover, the term carbocyclic as used throughout the specification and claims is intended to include both "unsubstituted carbocycles" and "substituted carbocycles", the latter of which refers to carbocyclic moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. Cycloalkyls can be further substituted, e.g., with the substituents described above. An "alkylaryl" moiety is an alkyl substituted with an aryl (e.g., phenylmethyl (benzyl)).

The term "aryl" as used herein, refers to the radical of aryl groups, including 5- and 6-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene, pyrrole, furan, thiophene, imidazole, benzoxazole, benzothiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Aryl groups also include polycyclic fused aromatic groups such as naphthyl, quinolyl, indolyl, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles", "heteroaryls" or

"heteroaromatics". The aromatic ring can be substituted at one or more ring positions with such substituents as described above, as for example, halogen, hydroxyl, alkoxy, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkylaryl, or an aromatic or heteroaromatic moiety. Aryl groups can also be fused or bridged with alicyclic or heterocyclic rings which are not aromatic so as to form a polycycle (e.g., tetralin).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure, even more preferably one to three carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths.

The terms "alkoxyalkyl", "polyaminoalkyl" and "thioalkoxyalkyl" refer to alkyl groups, as described above, which further include oxygen, nitrogen or sulfur atoms replacing one or more carbons of the hydrocarbon backbone, e.g., catenary oxygen, nitrogen or sulfur atoms.

The terms "polycyclyl" or "polycyclic radical" refer to the radical of two or more cyclic rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, hydroxyl, alkylcarbonyloxy, arylcarbonyloxy, alkoxycarbonyloxy, aryloxycarbonyloxy, carboxylate, alkylcarbonyl, alkoxycarbonyl, aminocarbonyl, alkylthiocarbonyl, alkoxyl, phosphate, phosphonato, phosphinato, cyano, amino (including alkyl amino, dialkylamino, arylamino, diarylamino, and alkylarylamino), acylamino (including alkylcarbonylamino, arylcarbonylamino, carbamoyl and ureido), amidino, imino, sulfhydryl, alkylthio, arylthio, thiocarboxylate, sulfates, sulfonato, sulfamoyl, sulfonamido, nitro, trifluoromethyl, cyano, azido, heterocyclyl, alkyl, alkylaryl, or an aromatic or heteroaromatic moiety.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are nitrogen, oxygen, sulfur and phosphorus.

It will be noted that the structure of some of the compounds of this invention includes asymmetric carbon atoms. It is to be understood accordingly that the isomers arising from such asymmetry (e.g., all enantiomers and diastereomers) are included within the scope of this invention, unless indicated otherwise. Such isomers can be obtained in substantially pure form by classical separation techniques and by stereochemically controlled synthesis.

Examples of suitable carbocyclic moieties include substituted or unsubstituted hydrocarbons, e.g., adamantyl; or substituted or unsubstituted aromatic compounds such as naphthyl, quinolyl, acridinyl, tetrahydroacridinyl, anthracenyl, benzopyrenyl, and the like. Large carbocyclic cage moieties such as porphyrins can also be used in the therapeutic compounds and methods of the invention. The carbocyclic moiety preferably has a steric bulk greater than the steric bulk of a phenyl group; certain compounds in which C is a phenyl group have been found to be ineffective glycolipid mimics.

In certain embodiments of the invention, acridine and adamantane derivatives, as well as those listed in the paragraph *supra* are not included.

In a preferred embodiment, the carbocyclic moiety includes a portion which can be coupled to the glycolipid receptor moiety, e.g., a carboxylic acid, amine or ester. Coupling can be effected by covalent, ionic, charge/charge interactions, etc. for attachment to the glycolipid receptor moiety. For example aminoadamantanes or aminoacridines can be coupled to the carboxyl group of the oxidized sphigosine moiety. Likewise, carboxyladamantanes or carboxylacrines, e.g., carboxylic acids, can be coupled to the amino group of a deacylated glycolipid.

The phrase "associated with a pathogenic microorganism" is intended to include, but is not limited to, those microorganisms, e.g., bacteria, which are pathogenic to the host subject as listed in Table I.

TABLE I

| Organism | Binding to $Gg_3$ and $Gg_4$ |
|---|---|
| Streptococcus pneumoniae | + |
| Streptococcus agalactiae (Gp. B.) | + |
| Branhamella catarrhalis | + |
| Chlamydia trachomatic | + |
| Chlamydia pneumoniae | + |
| Clostridium perfringens | + |
| Clostridium difficile | + |
| Staphylococcus aureus | + |
| Klebsiella pneumoniae | + |
| Borrelia burgdorferi | + |
| Haemophilus influenzae | + |
| Haemophilus parainfluenzae | + |
| Pseudomonas aeruginosa | + |
| Pseudomonas cepacia | + |
| Pseudomonas maltophilia | + |
| Neisseria gonorrhoeae | + |
| Nisseria meningitidis | + |
| Helicobacter pylori | + |
| Shigella dysenteriae | + |
| Shigella flexneri | + |
| Pasturella multocida | + |
| Coxiella burnetti | + |
| Mycobacterium tuberculosis | + |
| Mycobacterium avium-intracellulare | + |
| Salmonella typhymurium | + |
| Escherichia coli ATCC 6883 | + |
| Escherichia coli HB101/DH5a | + |
| Bacillus subtilis | |
| Escherichia coli K99 | |
| Listeria monocytogenes | |
| Vibrio cholera | |
| Mycoplasma sp. | |
| Streptococcus pyogenes | |

In another aspect, the invention pertains to methods of modulating interaction between a pathogenic microorganism and a glycolipid in a subject by administering to the subject a therapeutically effective amount of a therapeutic compound, such that interaction between a pathogenic microorganism and a glycolipid is modulated. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent as discussed *supra*.

The terms "modulate", "modulating" and "modulation" are intended to include preventing, eradicating, or inhibiting interaction between a pathogen and a glycolipid, e.g., in the context of the therapeutic methods of the invention. In another embodiment, the term modulate includes effects on SLTs, e.g., verotoxin, that diminishes the activity or production of the toxins(s). For example, the therapeutic compound can interact with the toxin(s) to inhibit proteolytic activity.

In yet another aspect, the present invention provides methods for treating a state characterized by the presence of a shiga-like toxin (SLT) in a subject by administering to a subject a therapeutically effective amount of a therapeutic compound, such that a state characterized by the presence of shiga-like toxin is treated. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

The language "state characterized by the presence of a SLT" is intended to include those diseases, disorders or conditions which have been associated with a toxin, e.g., an enterotoxin, produced by a pathogen, e.g., bacteria, in that the pathogen is directly or indirectly a causative agent of the disease, disorder or condition. The pathogen does not have to be the sole causative agent of the disease, disorder or condition but be merely responsible for causing some of the symptoms typically associated with the disease, disorder, or condition being treated. The pathogen can be the causative agent alone or at least one other agent can be involved in the state being treated. Examples include uncomplicated diarrhea, bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS), fluid electrolyte imbalances, anemia, renal failure and/or hypertension manifested by the presence of symptomatic responses, such as gastritis. (*Salmonella typhi*), food poisoning (*E. coli* O157:H7), bascillary dysentery (*Shigella dysenteria*), pneumonia ((Psuedomonas aerugenosa) and cholera (*Vivrio cholerae*). Preferred examples include those symptoms associated with *E. coli*.

Moreover, Hemolytic uremic syndrome (HUS) is the primary cause of acute pediatric renal failure. The majority of cases occur in children under three years of age but HUS may also occur in the elderly and occasionally in adults. HUS is defined by a triad of clinical symptoms: thrombocytopenia, hemolytic anemia and

microvasculopathy. Epidemiological studies within the last 15 years have established that HUS is caused primarily by gastrointestinal infection with verotoxin producing *E. coli*. (VTEC). Gastrointestinal infection with VTEC, primarily of the O157 H7 serotype, can cause hemorrhagic colitis (HC) which may progress to HUS. In these pathologies, systemic verotoxin targets the endothelial cells within the microvasculature of the gastrointestinal tract and the pediatric renal glomerulus. VTEC are not believed to be invasive and thus the clinical pathology is the result of translocation of verotoxin across the gastrointestinal barrier to the systemic circulation. Structural studies indicate that the verotoxin receptor glycolipid (globotriaosyl ceramide-$Gb_3$) is not present on the gastrointestinal epithelial cell surface and therefore the mechanism by which the toxin translocates from the GI tract is essentially unknown. Studies *in vitro* and in animal models however indicate that the attachment of the verotoxin producing *E. coli* organism to the host epithelial cell membrane may be intimately involved in the mechanism by which the toxin translocates. Similarly, attachment of the organism to the gastrointestinal host cell plasma membrane is an important virulence trait in the induction of diarrhea.

Verotoxins (or Shiga like toxin) comprise a family of subunit toxins which target the glycolipid globotriaosyl ceramide ($Gb_3$) expressed on the surface of sensitive cells.

The language "treating or treatment of the state characterized by the presence of an SLT" is intended to include the alleviation of or diminishment of at least one symptom typically associated with the state. The treatment also includes alleviation or diminishment of more than one symptom. Preferably, the treatment cures, e.g., substantially eliminates, the symptoms associated with the state.

In one aspect, the present invention pertains to compounds represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent. Synthesis of the compounds represented by the structure A-B can be accomplished by various approaches detailed as follows (For example, see also Sakac, D. *et al.* "Purification of the Testicular Galactolipid 3' Phosphoadenosine 5' Phosphosulfate Sulfotransferase" J. Biol. Chem. **267**:1655-1659 (1992); Lingwood C.A. "The Production of Glycolipid Affinity Matrices by Use of Heterobifunctional Crosslinking Reagents" J. Lipid Res. **25**:1010-1012 (1984); Lingwood C.A. and Taylor T. "Synthesis and Use of Galactolipid Sulfotransferase Substrate-analogue Affinity Probes" Biochem. Cell Biol. **64**:631-637 (1986) and Thong B. *et al.* "Anti-digoxin Antibodies: Lack of Specificity of Current Antisera. Preparation of New, Specific Antibody which Recognizes the Carbohydrate Moiety of Digoxin" Clin. Chem. **31**:1635-1631 (1985); and Boulanger, J. *et al.* Anal. Biochem. 217:1-6 (1994)).

The expression of surface adhesins or toxins, e.g., VTEC, specific for $Gg_3/Gg_4$ or PE allows for the accumulation of a lipid receptor-analog/antibiotic conjugate at the bacterial cell surface. Antibiotics which are active at the bacterial surface are coupled to derivatives of either $Gg_3/Gg_4$ or PE. These "receptocides" are bound by the bacterium and this accumulation results in the more efficient inhibition of bacterial membrane assembly. In addition, these receptocides function as anti-adherents to prevent the attachment of the pathogenic organism to host cells. Furthermore, any development of resistance due to the loss of such adhesin species can be avoided since loss of the adhesin, in order to avoid binding of the receptocide, can also result in the loss of ability to bind to host cells. Moreover, the lipid binding specificity is restricted to pathogenic organisms (VTEC) and thus would spare the beneficial commensal *E. coli* strains , in contrast to broad spectrum antibiotics.

In one method, $Gg_4$ can be prepared from GM1 and $Gg_3$ from GM2 ganglioside (both commercially available) by mild acetic acid hydrolysis to remove the sialic acid. For example, $Gg_4$ is first treated with aqueous base when the aminosugar is preferentially deacylated (since the lipid moiety is sequestered in micelles) and the free amine is alkylated, e.g., dimethylated. The ceramide of the dimethyl $Gg_4$ is then deacylated with alcoholic base and the free amine of the sphingosine base is coupled, for example, to the carboxyl group of an antibiotic, for example N-acetyl penicillin, as shown in Figures 1A and 1B (Schemes 1A and 1B).

In a second method, oxidative cleavage of the double bond in the sphingosine of glycosphingolipids affords a carboxylic acid ("glycosphingosinic" acid) derivative for coupling to amino containing antibiotics. Oxidation of the sphingosine double bond of glycolipids by ozonolysis has been previously described. This method can be improved by the use of $KMnO_4$ oxidation in the presence of a crown ether. In a preferred method, depicted in Figures 2A and 2B, the oxidation procedure utilizes catalytic amounts of $KMnO_4$ (plus a regeneration system to prevent $MnO_2$ precipitation in tertiary butyl alcohol (Schemes 2A and 2B). This method provides the advantages that i) tertiary butyl alcohol is not liable to $KMnO_4$ oxidation, ii) lack of precipitation prevents product loss by adsorption. This procedure also affords high yields (40-80%) of a single product.

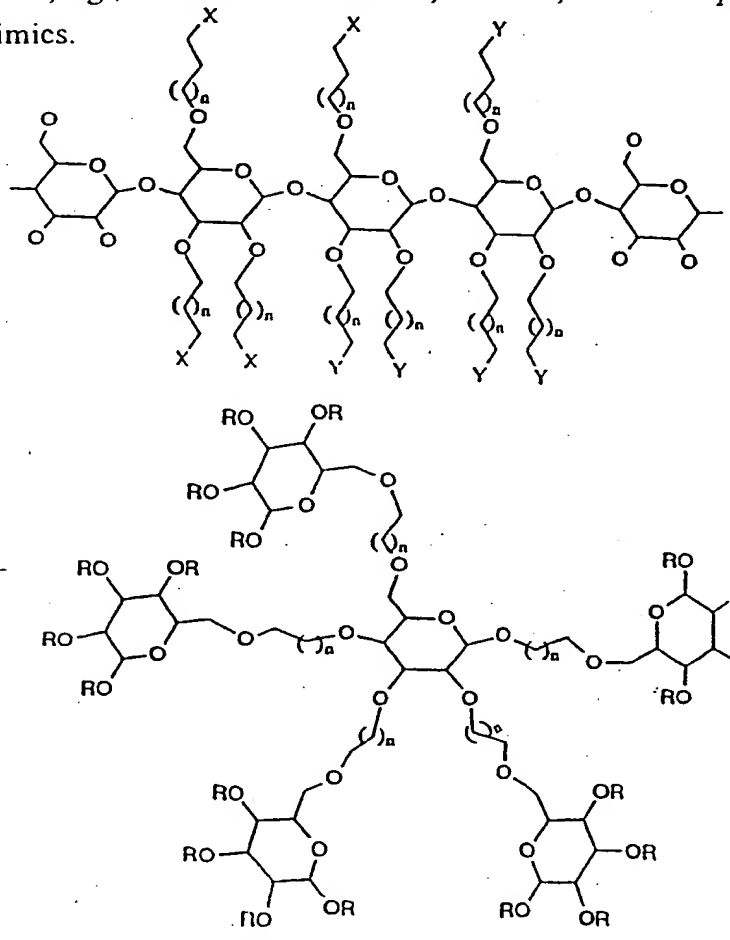
In another embodiment, the glycolipid is first deacylated to remove the fatty acid and the free amine is alkylated, e.g., methylated resulting in the dimethylation of the aminosugar in $Gg_3$. The sugar residues are then acetylated prior to oxidation of the sphingosine double bond as in Figures 2A and 2B (Schemes 2A and 2B).

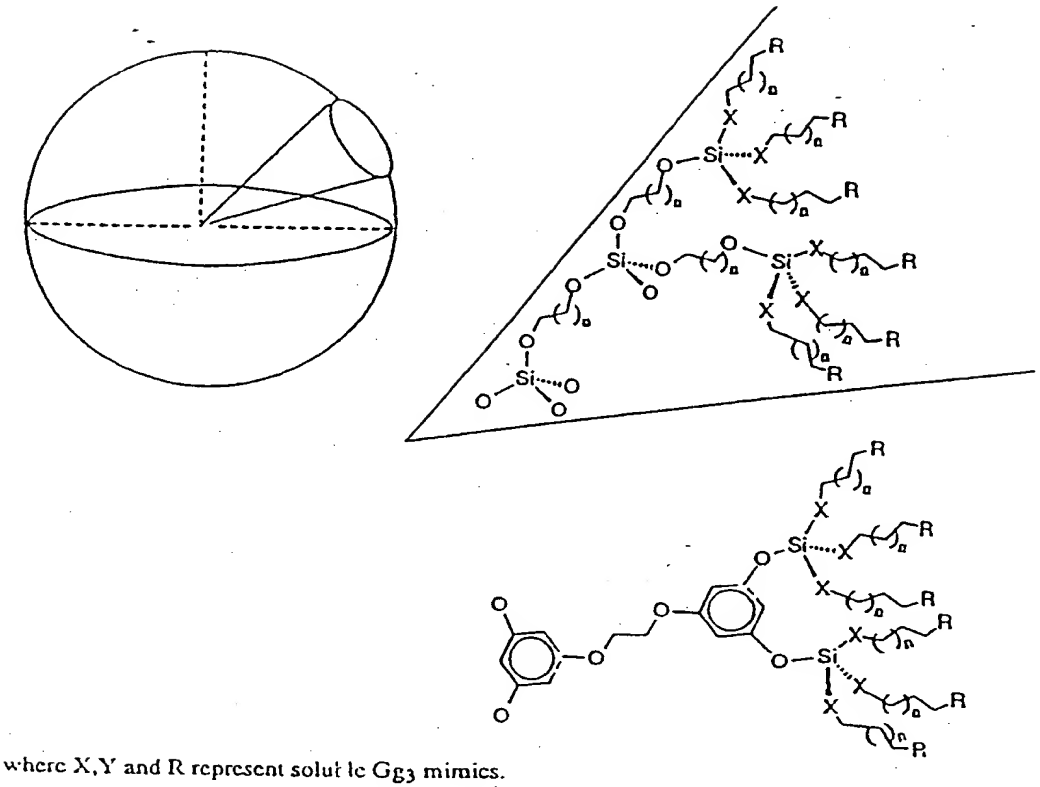
The carboxylic acid can be activated using procedures known in the art, for example, N-OH succinimide and coupled, using dicyclohexylcarbodiimide, to the amino function of an antibiotic as shown in Figure 3 (Scheme 3) (shown for $Gg_3$). The hydroxyl groups can be regenerated by deacetylation using triethylamine base.

Preferred embodiments include monoalkylated, dialkylated, monoarylated or diarylated deacylated glycolipids described by the above procedures. For example, the resultant amino functionality of the deacylated glycolipid can be treated with alkylating or arylating agents known in the art. Preferably, the amine is dialkylated or diarylated with lower alkyl groups, e.g., methyl, ethyl, propyl, or aryl groups whose steric bulk do not interfere with the bioreactivity of the resultant conjugate, e.g., benzyl, benzoyl, aryl.

It is possible to go beyond these initial receptocides and synthesize species which have several (including different) antibiotics coupled to a single carbohydrate (shown in Figure 4)(Scheme 4)). For example, the amino crosslinker (tertiary butylamine) can be added in large excess to the carboxyl $Gg_4$ derivative and coupled using dicyclocarbodiimide. After purification, the triamino $Gg_4$ can then be coupled to antibiotic(s) as discussed above.

Additionally, therapeutic compounds depicted below can be prepared by known coupling reactions, e.g., etherification reactions, where X, Y and R represent soluble $Gg_3$ or $Gg_4$ mimics.





where X,Y and R represent soluble $Gg_3$ mimics.

Derivatization of the amino group of PE may result in the loss of bacterial receptor activity. However, lyso-PE in which the C3 position which contains an ether linked fatty acid and a free hydroxyl group which is present at C2 andis strongly bound by some SLTs, e.g., VTEC. Accordingly, a strategy depicted in Figure 5 (Scheme 5) demonstrates that coupling of an antibiotic species to the C2 OH of lyso-PE generates PE/antibiotic receptocides which can selectively target SLTs as opposed to commensal GI *E. coli* strains. The procedure involves protecting the primary amine, followed by oxidation of the glycerol alcohol to give the corresponding ketone. Coupling of an amino antibiotic by reductive amination and finally deprotection of the amine of ethanolamine results in the final PE receptoside.

The structures of the receptocides made can be determined by FAB mass spectrometry, proton NMR as well as those techniques known to persons of skill in the art.

It is believed that the availability of adhesin targeted antibiotics should allow the administration of bactericidal doses which represent significantly lower antibiotic doses when considered on a molar basis. The binding of the receptocides by the SLTs, e.g., VTEC, result in the concentration of the antibiotic at the bacterial cell surface for more efficient inhibition of membrane assembly. Judicious selection of antibiotics with activity against SLTs may result in the generation of new potent treatments for the effective and selective elimination of SLTs from the subject, e.g., human, GI tract.

The topology of the adhesins on the SLTs, e.g., VTEC, surface may not optimally correspond to the surface location of the antibiotic binding proteins (transpeptidases) necessary for antibiotic inhibition of bacterial membrane assembly. Therefore, the introduction of an appropriate space group between the receptor and the antibiotic may further improve antibiotic efficacy by optimizing the matching of adhesin and antibiotic-binding-protein topology. Suitable spacer groups are known in the art and can include anhydrides, haloalkylamines and the like.

In another aspect, the present invention pertains to pharmaceutical compositions which include a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent, described *supra*, and a pharmaceutically acceptable carrier. These pharmaceutical compositions are useful in treatment of glycolipid mediated states and for modulating interaction(s) between a pathogenic microorganism and a glycolipid in a subject.

The phrase "pharmaceutically acceptable carrier" as used herein means a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a compound(s) of the present invention within or to the subject such that it can performs its intended function. Typically, such compounds are carried or transported from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and

aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain embodiments of the present compounds can contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable acids. The term "pharmaceutically acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, e.g., Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19).

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically acceptable salts with pharmaceutically acceptable bases. The term "pharmaceutically acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically acceptable metal cation, with ammonia, or with a pharmaceutically acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like.

The term "pharmaceutically acceptable esters" refers to the relatively non-toxic, esterified products of the compounds of the present invention. These esters can be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form or hydroxyl with a suitable esterifying agent. Carboxylic acids can be converted into esters *via* treatment with an alcohol in the presence of a catalyst. Hydroxyl containing derivatives can be

converted into esters *via* treatment with an esterifying agent such as alkanoyl halides. The term is further intended to include lower hydrocarbon groups capable of being solvated under physiological conditions, e.g., alkyl esters, methyl, ethyl and propyl esters. (See, for example, Berge et al., *supra.*)

The invention further contemplates the use of prodrugs which are converted *in vivo* to the therapeutic compounds of the invention (see, e.g., R.B. Silverman, 1992, "The Organic Chemistry of Drug Design and Drug Action", Academic Press, Chp. 8). Such prodrugs can be used to alter the biodistribution (e.g., to allow compounds which would not typically enter the reactive site of the protease) or the pharmacokinetics of the therapeutic compound. For example, a carboxylic acid group, can be esterified, e.g., with a methyl group or an ethyl group to yield an ester. When the ester is administered to a subject, the ester is cleaved, enzymatically or non-enzymatically, reductively or hydrolytically, to reveal the anionic group. An anionic group can be esterified with moieties (e.g., acyloxymethyl esters) which are cleaved to reveal an intermediate compound which subsequently decomposes to yield the active compound. In another embodiment, the prodrug is a reduced form of a sulfate or sulfonate, e.g., a thiol, which is oxidized *in vivo* to the therapeutic compound. Furthermore, an anionic moiety can be esterified to a group which is actively transported *in vivo*, or which is selectively taken up by target organs. The ester can be selected to allow specific targeting of the therapeutic moieties to particular reactive sites, as described below for carrier moieties.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for oral, nasal, topical, transdermal, buccal, sublingual, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage

form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; solution retarding agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; absorbents, such as kaolin and bentonite clay; lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the active compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given by forms suitable for each administration route. For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administration is preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systematically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 200 mg per kilogram of body weight per day, more preferably from about 0.01 to about 150 mg per kg per day, and still more preferably from about 0.2 to about 140 mg per kg per day.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical composition.

In still another aspect, the present invention pertains to packaged therapeutic compositions for treating a glycolipid mediated state in a subject. The packaged therapeutic compositions include a container for holding a therapeutically effective amount of a therapeutic compound for treating a glycolipid mediated state in a subject and instructions for using the therapeutic composition for treating the glycolipid mediated state. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

In one aspect, the present invention pertains to packaged therapeutic compositions for modulating interaction between a pathogenic microorganism and a glycolipid. The packaged therapeutic composition includes a container for holding a therapeutically effective amount of a therapeutic compound for modulating interaction between a pathogenic microorganism and a glycolipid in a subject and instructions for using the therapeutic composition for modulating interaction between the pathogenic microorganism and the glycolipid. The therapeutic compound is represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

Since pathogenic *E. coli* provide the basic etiology of states characterized by the presence of SLTs, e.g., both HC and HUS, it was initially considered that antibiotic

treatment would be an effective therapy for these microvascular diseases. Clinical experience suggested that this was not the case. Indeed antibiotic treatment may exacerbate rather relieve symptoms. Studies have indicated that use of "appropriate" antibiotics (ampicillin, amoxycillin) is associated with a lack of progression to HUS without significant worsening of the diarrheal disease. However, use of "inappropriate" antibiotics is associated with higher incidence of HUS in shigellosis

Adverse antibiotic effects may be due to two factors: firstly that at the first appearance of SLT symptoms requiring medical attention (blood in stools), the pathogenic organisms in the stool are declining in number. Thus the major effect of antibiotics given at this time will be to remove commensal organisms which likely have a protective effect. Secondly, it has been proposed that antibiotic treatment may induce pathogenic lysis and thereby release a bolus of intracellular SLTs from dying organisms. In this regard it has been shown *in vitro* that treatment of pathogenic strains with subinhibitory concentrations of trimethoprim-sulfamethoxazole did in fact increase the release of SLTs.

Advantages of the invention include i) early diagnosis of SLTs, so that receptocide therapy can be initiated prior to the production of significant levels of SLTs, e.g., verotoxin, within the GI tract and ii) methods to target antibiotics to the pathogenic microorganism and not commensal GI organisms.

Several rapid ELISAs (including a receptor based ELISA (Verotest™) are now in clinical trials for detection of VT in stools (Donohue-Rolfe, A. *et al.* "Enzyme-linked Immunosorbent Assay for Shigella Toxin" J. Clin. Microbiol. 24:65-68 (1986) and Basta, M. *et al.* "Sensitive Receptor-specified Enzyme-linked Immunosorbent Assay for Escherichia coli Verocytotoxin" J. Clin. Microbiol. 127:1617-1622 (1989)). Measurement of SLTs in stools has been established as the most effective means of diagnosing SLT infections and the availability of rapid automated ELISAs will allow screening of diarrhea cases. With diagnosis of SLT induced diarrhea, receptocide treatment prior to overt pathological lesions, becomes feasible. Indeed, with this advance in diagnosis, development of novel treatment regimes becomes mandatory.

The invention is further illustrated by the following examples which in no way should be construed as being further limiting. The contents of all references and published patent applications, cited throughout this application are hereby incorporated by reference. It should be understood that the models used throughout the examples are accepted models and that the demonstration of efficacy in these models is predictive of efficacy in humans.

## EXPERIMENTAL

### *MATERIALS AND METHODS*

*MATERIALS*:

Solvents - dichloromethane (DCM), *tert*-butyl alcohol ($^{t}$BuOH), *iso*-propyl alcohol ($^{iso}$PrOH), 1,2-dichloro ethane (DCE), pyridine (Py), diethyl ether ($Et_2O$), benzene (Bz), methanol (M), chloroform (C), acetonitril (AcCN) and acetone (A) - were purchased from either Caledon (Georgetown, Ontario) or Aldrich (Milwaukee, WI) and ethanol (EtOH) from Commercial Alcohols Inc. (Brampton, Ontario). Reagents were purchased from the following suppliers: Caledon - trifluoroacetic anhydride, $K_2CO_3$, sodium cyanoborohydride ($NaBH_3CN$), triethylamine ($Et_3N$); Aldrich - 37% aqueous formalin solution, 0.5N $H_2SO_4$ solution, trichloroacetic anhydride, acetic anhydride, diphenyl succinimidyl phosphate (PNHS); BDH (Toronto, Ontario) - ANALAR $KMnO_4$, ANALAR $NaHSO_3$, 30% $H_2O_2$; Sigma (St. Louis, MO) - dimethysulfoxide (DMSO), oleic anhydride ($C_{36}H_{66}O_3$), erucic anhydride ($C_{44}H_{82}O_3$), 4-chloro-1-naphthol; Fluka - bissuccinimidyl oxalate (OxNHS) and from Fisher Scientific (Unionville, Ontario) - *meta*-$NaIO_4$. Chromatographic materials - Silica gel, TLC, HPTLC and aluminum backed nanosilica plates (alugram NanoSIL GI $UV_{254}$, Macherey & Nagel) - were supplied by Caledon. Reverse phase C-18 cartridges were obtained from Waters (Mississauga, Ontario) and molecular sieves, 4Å from Fisher. Centricon-30 centrifugal concentrators were purchased from Amicom®.

Solvents were dried by storing over activated (~120° C for 16 hrs) molecular sieves. Solvent systems are given in volume ratios. Crown ether (10 g) was recrystallized from a hexane (4 to 5 mL) solution at -20° C, washed with cold (-20° C) hexanes (1 mL) and dried at 40° C under a stream of $N_2$.

BSA (99%, essentially fatty acid free) was purchased from Sigma. Recombinant gp120 was purchased from Intracell (CA), anti-human IgG horse radish peroxidase conjugate from Bio-Rad and human sera from HIV patients containing anti-gp120 antibodies was a gift from Dr. S. Read, Division of Infectious Disease, HSC.

*Glycosphingolipids*:

Globotetraosyl ceramide, $Gb_4$•C, Globotriaosyl ceramide, $Gb_3$•C and Lactosyl ceramide, Lac•C were purified from human kidney (Boyd, B. and Lingwood, C. A. (1989) *Nephron* 51, 207-210) and Forssmann, $Gb_5$•C was purified from sheep blood (Ziolkowski, C. H., Fraser, B. A. and Mallette, M. F. (1975) *Immunochemistry* 12, 297-

302) and monosialylganglioside, $GM_1$•C was purified from bovine brain (Yamakawa, T., Irie, R. and Iwanaga, M. (1960) *J. Biochem.* 48, 490-497) according to previously published procedures. Galactocerebroside, Gal•C was purchased from Sigma. Gangliotetraosyl ceramide, $Gg_4$•C was prepared by acid hydrolysis of $GM_1$•C with 1 M acetic acid at 80° C for 1 hour (Head, S., Ramotar, K. and Lingwood, C. A. (1990) *Infect. Immun.* 58, 1532-1537). De-Nacylated derivates, $Gb_3$•S, Gal•S (phychosine) were prepared by saponification at 102° C with 1 M methanolic NaOH for 3 hours (Head *et al.* (1990)).

## *METHODS*

### *Synthesis of NN-dimethylated Derivatives: $Gb_3$•SNNMe$_2$ (Galα1→4 Galβ1→4Glcβ1→sphingosineNNMe$_2$), Gal•SNNMe$_2$ (Galβ1→sphingosineNNMe$_2$)*

To a solution of 1 mg of deacylated GSL (approximately, 2 μmol for monosaccharide, 1.5 μmol for disaccharide and 1.25 μmol for trisaccharide deacyl GSLs) in methanol (0.5 mL), 40 μL of 37% aqueous formaldehyde solution (15 mg of formaldehyde, 500 μmol) and 100 μL of 0.32 M methanolic solution of $NaCNBH_3$ (prepared by dissolving 20 mg of $NaCNBH_3$ in 1 mL of dry MeOH) were added (Borch, R. F., Bernstein, M. D. and Durst, H. D. (1969) *J. M. C. S.* 93, 2897-2904; Means, G. E. and Feeney, R. E. (1995) *Anal. Biochem.* 224, 1-16). After stirring, the reaction mixture for 16 hours at room temperature (25° C), methanol was removed under $N_2$ and the remaining solid was the dissolved, by sonication, in 5 mL of distilled water. The resulting suspension was passed through a C-18 reverse phase cartridge, washed with 20 mL of water and eluted with 20 mL of methanol. The yield of methylated product was >90% by TLC. TLC showed that the methylated compound has a reduced mobility compared to the deacyl forms. The $R_f$ values for Gal•S and Gal•SNNMe$_2$ are 0.80 and 0.75 in $CHCl_3$:MeOH:$H_2O$; 60:35:8 or 0.38 and 0.31 in $CHCl_3$:MeOH:$H_2O$; 65:25:4 respectively. Positive ion mass spectroscopic data, (m/z): Gal•SNNMe$_2$, FAB, 489, (M+H); $Gb_3$•SNNMe$_2$, ES, 814 (M+H), 836 (M+Na).

*Synthesis of N-trihaloacetyl Derivatives: Gal•SNTfa (Galβ1→sphingosineNTfa), Gal•SNTca (Galβ1→sphingosineNTca)*

Acetylating reagents, N-acetyl imidazole and N-trihaloacetyl imidazole, were prepared by adding a DCM solution of anhydride - for example $(CL_3CO)_2O$ (0.85 g, 2.7 mmol) dissolved in DCM (2mL) and the resulting solution was divided in 3 portions and added at 15 minute intervals, to an imidazole (0.41 g, 6.0 mmol) suspension in DCM (3 mL). The reaction mixture was stirred for 2 hours and was assumed to be approximately a 0.5 M solution of the imidazole derivative.

A solution of the imidazole derivative was added to a DCM suspension of GSL•S (1 mg/mL). For example, N-trichloroacetyl imidazole solution (20 µL, 10 µmol) was added to a suspension of Gal•S (3 mg in 3 mL of $CH_2Cl_2$, 6 µmol), and the reaction was monitored by TLC ($CHCl_3$:MeOH:$H_2O$; 70:30:2). Appearance of many orcinol positive products suggested some degree of acylation of OH groups. Once the GSL•S was consumed, DCM was removed under a stream of $N_2$, a solution of $Et_3N$:MeOH:$H_2O$; 2:6:10 (0.5 mL/mg of GSL) was added and incubated at RT and the reaction was monitored every 30 minutes by TLC ($CHCl_3$:MeOH:$H_2O$; 70:30:2). Once all the orcinol positive species collapsed to a single band, the reaction mixture was dried under a stream of $N_2$, redissolved in DCE and loaded on to a silica column (0.5 X 6 cm, in DCE) and eluted with $CHCl_3$:MeOH; 98:2 (batch elution, 25 mL) and then with $CHCl_3$:MeOH:$H_20$; 80:20:2 (10, 3 mL fractions were collected). The estimated product yield by TLC was >90%.

*Synthesis of Gal•C$^{oleic}$ (Galβ1→sphingosineN-oleic) and Gal•C$^{urecic}$ (Galβ1→sphingosineN-urecic) Homologues*:

To a solution Gal•S (2 mg, 4 µmol) in dry pyridine (2 mL) an excess of the anhydride (approximately 5 mg, corresponding to 9 µmol and 8 µmol for oleic and erucic anhydrides respectively) was added and stirred at 37° C for 18 hours. Pyridine was removed under a stream of $N_2$ and the residue was treated with 1 M methanolic NaOH (2 mL) for 5 hours at 25° C, neutralized with 1 M HCl (2 mL), diluted with water (5 mL), the aqueous phase extracted three times with 5 mL portions of $Et_2O$ and the combined extracts were dried. The crude extract was then dissolved in DCM (1mL) and loaded on to a silica column (0.5 X 10 cm in $CHCl_3$:MeOH; 98:2). The free fatty acids were eluted with DCE:$^{iso}$PrOH; 85:15 (20mL) and the product was eluted with

$CHCl_3$:MeOH:$H_2O$; 80:20:2 (6, 4 mL fractions were collected). The estimated yield by TLC was >95%.

***Synthesis of Peracetylated Derivatives: $Gal(OAc)_4$•C(OAc), $Gal(OAc)_4$•$C^{oleic}$(OAc), $Gal(OAc)_4$•$C^{urecic}$(OAc), $Lac(OAc)_7$•C(OAc), $Gb_3(OAc)_{10}$•C(OAc), $Gb_4(OAc)_{12}$•C(OAc), $Gg_4(OAc)_{12}$•C(OAc), $Gb_5(OAc)_{14}$•C(OAc), $GM_1(OAc)_{16}$•C(OAc)$_1$, $Gal(OAc)_4$•SNTfa(OAc), $Gal(OAc)_4$•SNTca(OAc), $Gal(OAc)_4$•SNAc(OAc), $Gal(OAc)_4$•$SNNMe_2$-(OAc), $Gb_3(OAc)_{10}$•SNAc(OAc), and $Gb_3(OAc)_{10}$•$SNNMe_2$(OAc)***

Method A Suitable for natural, NAc and $NNMe_2$ derivatives. A mixture of 1:2 acetic anhydride and pyridine (1 mg/mL of lyso GSL) was added to a dried sample of natural GSL, GSL S or GSL SNNMe2 and stirred at 37° C. The reactions were monitored every 30 minutes by TLC using DCE:$^{iso}$PrOH; 80:15 as solvent system, and upon completion, dried under a stream of $N_2$.

Method B Suitable for the preparation of $NTca(OAc)_n$ and $NTfa(OAc)_n$ derivatives. A mixture of 2:1 trifluoroacetic anhydride and glacial acetic acid (1 mL/mg of glycolipid) was added to a dried sample of NTfa oor NTca, GSL derivatives and stirred at 25° C. The reactions were monitored every 30 minutes by TLC using DCE:$^{iso}$PrOH; 80:15 as solvent system, and upon completion, dried under a stream of $N_2$.

The peracetylated crude was dissolved in DCE (1 mL) and loaded on to a silica column (for 3 mg, 0.5 X 5 cm in DCE) and eluted DCM:MeOH; 25:Y, Y being methanol which was varied from 100 µL in increments of 100 µL, where for each case 6, 4 mL fractions were collected. It is noteworthy that the mobility of most of the peracetylated derivates during column chromatography vary significantly with the degree of silica gel activation, and concomitant changes of the solvent ratio of the eluent may be necessary.

***Oxidation Reactions***:

***Oxidation Using $MKnO_4$/$^t$BuOH/$H_2O$/$NaIO_4$/$K_2CO_3$ System***:

Reagent: A 2:1 mixture of $^t$BuOH:$H_2O$. Solutions of $NaIO_4$ (0.4 M), $K_2CO_3$ (0.25 M) and $KMnO_4$ (0.05 M). Quenching solution: A5:1 mixture of 0.24 M $NaHSO_3$ solution and 0.5 M $H_2SO_4$ solution.

Peracetylated glycolipid (0.5 mg; depending on the GSLs this might vary from 1 to 0.3 μmol) was dissolved in tBuOH/H2O (500 μL) and solutions of NaIO4 (30 μL, 10 μmol), K2CO3 (10 μL, 2.5 μmol) and KMnO4 (15 μL, 0.75 μmol) were added in the given sequence. The resulting purple, turbid mixture was stirred at 37° C for 2 to 3 hours, depending on the GSL. If purified peracetylated derivatives are employed, the overall color of the reaction mixture should not diminish during the course of the reaction. However, if diminishing purple color is observed (due to the presence of impurities) with concomitant formation of brown $MnO_2$, additional aliquots (5 μL) of $KMnO_4$ solution should be added. The reaction was quenched by the addition of 1.5 mL of quenching solution and 1 mL of water and the resulting colorless solution was extracted three times with 5 mL portions of $Et_2O$. If, during the ether extraction procedure, any yellow color is observed, the combined organic phase is extracted with 1 mL of quenching solution. The combined organic phase is washed twice with 1 mL portions of water and dried under $N_2$ at 25° C. Residual water present in the crude product can be removed by adding 1 to 2 mL absolute EtOH and removing under $N_2$. The product was dissolved in 2:1 DCM:MeOH and stored below -20° C.

Deacyl GSLs (0.3 mg) were dissolved in $^tBuOH/H_2O$ (500 μL) and solutions of $NaIO_4$ (30 μL, 10 μmol), $K_2CO_3$ (10 μL, 2.5 μmol) and $KMnO_4$ (15 μL, 0.75 μmol) were added in the given sequence. The resulting puple, turbid mixture was stirred at 37° C for 1 to 2 hours, depending on the type of GSL•S. To isolate peracetylated serine oligosaccharide acids which have hydrophobic amino substituents like benzoyl or Tfa, the workup procedure employed to isolate ceramidic acids is applicable. However, in the case of less hydrophobic substituent like acetyl or charged groups like $NMe_2$, the reaction was quenched by the addition of an excess of solid $NaHSO_3$ (50 mg), gives a colorless, or occasionally pale yellow, suspension. This suspension is then dried on a rotary evaporator and extracted with C:M:W' 80:20:2 (15 to 20 mL) which in turn was passed through a silica column (0.5 cm X 4 cm in the same solvent) to remove most of the salts.

Deprotection of the ceramidic acids or the serine oligosaccharide acids were carried out by treating 0.5 mg of dry ceramidic acid with 1 mL of triethyl amine solution ($Et_3N$:MeOH:$H_2O$; 2:6:10) at 37° C for 2 to 3 hours. The reaction mixture was dried under $N_2$ and the residue redissolved in 2:1 DCM:MeOH.

*Oxidation Using $KMnO_4$/Crown Ether and $KMnO_4$/Aetone Systems*

A slightly modified version of the procedure described by Young *et.al.* (Young, J., W. W., Laine, R. A. and Hakomori, S. (1979) *J. Lipid Res.* 20, 275-278), was employed. Instead of forming the $KMnO_4$•crown-ether complex *in situ* by adding solid $KMnO_4$ and crown-ether to a benzene solution of GSL precursor, the complex was made separately and added to the reaction.

Reagent: To a solution of dicyclohexyl-18-crown-6 in benzene (0.05 M) an excess of $KMnO_4$ (approx. 20 mg) was added and sonicated for 15 minutes. The mixture was then centrifuged and the purple supernatant was assumed to contain approximately 0.05 M solution of 1:1 adduct of $KMnO_4$•Crown-ether. This solution should be freshly prepared since it slowly deposits $MnO_2$ upon standing.

Oxidation of $Gal(OAc)_4$•C(OAc) by $KMnO_4$ in acetone was carried out according to the published procedure (MacDonald, D. L., L., P. and Hakomori, S. I. (1980) *J. Lipid Res.* 21, 642-645), except product purification was similar to the isolation of ceramidic acids described in the new method.

*Mass Spectroscopic Analyses*

Permethylation of ceramidic acid, $Gg_4$•C-$^S$COOH was performed according to published methods (Fan, J. Q., Huynh, L. H., Reinhold, B. B., Reinhold, V. N., Takegawa, K., Iwahara, S., Kondo, A., Kato, I. and Lee, Y. C. (1996) *Glycoconj. J.* 13, 643-648). To approximately 100 µg of dried $Gg_4$•C-$^S$COOH, a suspension of NaOH in DMSO (100 µL of 5% suspension) was added and incubated at 25° C for 1 hour. To this 100 µL of MeI was added and after allowing to stand at 25° C for 1 hour, unreacted MeI was removed by passing a stream of N2 for 15 minutes, 3 mL of water and 5 mL of DCM were added and the organic phase was washed three times with 3 mL portions of water and dried. The ES spectra were recorded on a SCIEX API III spectrometer and FAB on VG ZAB-SE using standard conditions (Aspinall, G. O., Monteiro, M. A., Pang, H., Walsh, E. J. and Moran, A. P. (1996) *Biochemistry* 35, 2489-2497).

*Galactosyl Ceramide/BSA Coupling Reaction and gp120 Binding*

The precursors derived from Gal•C, Gal•C-$^S$COOH, Gal•S-COOH and Gal•S of were coupled to BSA. Prior to coupling, the oxidized products, $GAL(OAc)_4$•C(OAc)-$^S$COOH and $GAL(OAc)_4$•S(OAc)-COOH (500 µg) were deprotected with triethyl amine solution and dried. The crude deprotected acids, Gal•C-$^S$COOH and Gal•S-COOH were

dissolved (0.5 mL of C:M:W; 80:20:2) and loaded on to a silica column (0.5 X 2 cm) and eluted, first with C:M:W; 80:20:2 (5 mL) and the with MeOH (6 mL). The Gal•C-$^{S}$COOH and Gal•S-COOH precursors were converted to the corresponding NHS derivatives by treating (dissolved in 5:1 AcCN:$Et_3N$ to give a final concentration of 1 mg/mL) with PNHS (Giambattista, M. D., Nyssen, E., Pecher, A. and Cocito, C. (199) *J. Biol. Chem.* 29, 9203-9211; Ogura, H., Nagai, S. and Takeda, K. (1980) *Tetrahedron Lett.* 21, 1467-1468) or OxNHS (Kometani, T., Fitz, T. and Watt, D. S. (1986) *Tetrahedron Lett.* 27, 919-922) (2 to 4 equivalence) at 37° C for 3 hours. Then the solvent was removed under a flow of $N_2$ and a solution of BSA in PBS (2 mg/mL stock, 1:1 w/w ratio of BSA:acid) was added and stirred at 37° C for 24 hours. Psychosine (Gal•S) was coupled by adding a solution of BSA (2 mg/mL in PBS) in 1:1 ratio (w/w) and the resulting mixture was treated with the coupling reagent (2 to 4 equivalence) and stirred at 37° C for 24 hours. The reaction mixture was transferred into a centricon-30 and washed 3 times with 1mL portions of PBS. These BSA conjugates were separated by SDS-PAGE, transferred to nitrocellulose or adsorbed directly on to nitrocellulose and tested for recombinant gp120 (rgp120) binding.

The nitrocellulose membranes were blocked with 5% milk powder, 0.05% tween-20 in 10mM TBS for 2 hours. Rinsed 3 times (10 to 15 minutes each) with 0.05% tween-20 in 10 mM TBS and incubated with rgp120, 1:1000 dilution in 3% milk powder in 10 mM TBS for 2 hours. Washed as described above and incubated with human HIV serum, 1:50 dilution in 5% milk powder, 0.05% tween-20 in 10 mM TBS for 2 hours. After rinsing as described above, the blots were incubated with the secondary antibody (anti-human IgG horse radish peroxidase conjugate), 1:1000 dilution in 5% milk powder, 0.05% tween-20 in 10 mM TBS for 45 minutes. Finally the blots were rinsed 3 times with 0.05% tween-20 in 10 mM TBS and a fourth rinse with only 10 mM TBS. Binding was visualized according to previously published procedure (Lingwood, C. A., Law, H., Richardson, S., Petric, M., Brunton, J. L., DeGrandis, S. and Karmali, M. (1987) *J. Biol. Chem.* 262, 8834-8839), by treating with 4-chloro-1-naphthol (3 mg/mL freshly prepared solution in methanol mixed with 5 volumes of 10 mM TBS and 1:1000 dilution of $H_2O_2$.

Analysis of approximately 5 µg of each conjugate by "western" or by dot blot gp120 overlay, showed that the conjugates (Gal•C-$^{S}$COHN)$_n$BSA and (Gal•C-SNHOC)$_n$BSA showed similar binding to gp120, whereas no binding was observed for (Gal•SNAc-COHN)$_n$BSA conjugate (Figure 6). In (Gal•C-$^{S}$COHN)$_n$BSA and (Gal•C-SNHOC)$_n$BSA conjugates the respective binding moieties retain an hydrocarbon chain - the sphingosine base or the fatty acyl chain, respectively. Thus,

even if the galactose residue is presented in a multivalent form, the galactosyl serine oligosaccharide conjugate (Gal•SNAc-COHN)$_n$BSA, is not recognized by gp120, indicating the presence of at least one of the hydrocarbon chains is essential for binding. This is consistent with the lack of binding inhibition by free galactose (Bhat, S., Spitalinik, S. L., Gonzalez-Scarano, F. and Silberberg, D. H. (1991) *Proc. Natl. Acad. Sci. USA* 88, 7131-7134). Influenza A virus also binds to galactosyl ceramide or sulfatide (Suzuki, T., Sometani, A., Yamazaki, Y., Horiki, G., Mitzutani, Y., Masuda, H., Yamada, M., Tahara, H., Xu, G., Miyamoto, D., Oku, N., Okada, S., Kisio, M., Hasagawa, A., Ito, T., Kawaoka, Y. and Suzuki, Y. (1996) *Biochem. J.* 318, 389-393), and again the lipid moiety is important for binding. The BSA conjugate we have made may therefore also bind this virus.

***Lipid Receptor Binding Specificity of Verotoxin Producing E. coli***

Studies have established that verotoxin producing E. coli (six strains tested) specifically bind to Gg$_4$ and PE. Sulfatide is also bound. Comparison of this binding specificity with enteropathogenic E. coli (six strains tested) demonstrated that receptor activity of these lipids for this class of E. coli is considerably less than for VTEC strains (Figure 7). In comparison, binding of commensal and non-pathogenic laboratory E. coli strains to these lipid structures *in vitro* appears to correlate with bacterial pathogenicity.

E. coli adherence is defined by a two stage process; firstly as a loose attachment to host cells, and secondly, a more consolidated tight attachment involving the intimin protein. In EPEC this initial loose attachment is mediated by the bundle forming pilus. However, in VTEC the mechanism of such initial host cell interaction is unknown. It is believed that the selective binding of Gg$_4$ and PE by VTEC may function in place of the bfp-mediated attachment.

***Antibiotic/glycolipid conjugates***

Dimethyl deacyl Gg$_3$ was coupled to N-acetyl penicillin (as indicated for Gg$_4$ in Figure 1B) and tested the efficacy of the conjugate ('receptocide') on the growth of *Hemophilus influenzae* (which has been shown to bind Gg$_3$ *in vitro*). N-acetyl penicillin is considered a poor antibiotic for *Hemophilus influenzae* and was found by agar gel diffusion to be virtually ineffective against this organism under the conditions used. However the "receptocide" was found to be almost as effective to inhibit *H. influenzae*

as penicillin. Thus targeting the antibiotic by coupling to $Gg_3$ had a major effect (several orders of magnitude) to increase antimicrobial efficacy.

In a second study, globotetraosyl ceramide ($Gb_4$) was coupled to ampicillin via oxidation of the glycolipid as shown in Figure 3 for $Gg_3$. Uropathogenic *E. coli* express P pili to mediate binding to globoseries glycolipids. In Figure 8 it can be seen that the $Gb_4$-ampicillin conjugate was effective to inhibit the growth of an uropathogenic *E. coli* (more than ampicillin itself) but was not effective for a VTEC strain (which does not bind $Gb_4$). It was observed that the VTEC was less sensitive to $Gb_4$-ampicillin than to ampicillin, suggesting that such receptocides may be even more specific than expected. The uncoupled glycolipid demonstrated no inhibitory effect.

These results demonstrate the validity of this approach to treat SLTs and generate targeted antibiotics, such as receptocides which show enhanced antibacteriocidal activity according to bacterial binding specificity.

***Inhibition of VT1 binding to $Gb_3$ Phospholipid Bilayer***

It has been shown that the binding of verotoxin to synthetic galabiose glycolipid species was markedly dependent on the nature of the lipid moiety (Boyd, B. *et al.* "Lipid Modulation of Glycolipid Receptor Function: Presentation of Galactose α1-4 galactose Disaccharide for Verotoxin Binding in Natural and Synthetic Glycolipids" Eur. J. Biochem. **223**: 873-878 (1994) and Pellizzari, A. *et al.* "Binding of Verocyctotoxin 1 to Its Receptor is Influenced by Differences in Receptor Fatty Acid Content" Biochem. **31**:1363-1370 (1992)). Moreover, binding of verotoxin to human renal $Gb_3$ was found to be a function of the fatty acid heterogeneity within the ceramide moiety (Pellizzari, A. *et al.* "Binding of Verocyctotoxin 1 to Its Receptor is Influenced by Differences in Receptor Fatty Acid Content" Biochem. **31**:1363-1370 (1992) and Kiarash, A. *et al.* J. Biol. Chem. **269**:11138-11146 (1994)). It has also been shown that binding of two members of the verotoxin family (VT1 and VT2c), which show a thousand-fold difference in cytotoxicity *in vitro* dependent on B subunit/receptor binding, preferentially bound different fatty acid-$Gb_3$ isoforms. An interesting observation was made at this time in that these two toxins did not compete together for glycolipid binding when binding the preferred fatty acid isoform. This problem was resolved in part in by molecular modeling studies in which it was proposed there were in fact two $Gb_3$ binding sites per B subunit monomer. It was therefore suggested that the different toxins preferentially used these different receptor binding sites and that different $Gb_3$ conformations could be preferentially accommodated in two different sites and such

conformations were defined by the lipid content of the globotriaosyl ceramide. First it is believed that not only does the presence of the glycolipid result in a million-fold increase in binding affinity but that the lipid moiety in some way influences the relative conformation of the carbohydrate to fit either one or the other binding site on the toxin. It is this effect of the lipid moiety on the conformation of the oligosaccharide that is successfully mimicked by the present invention. The concept includes that the molecule contains a truncated glycolipid (glycolipid acid) in which both the fatty acid has been removed and the sphingosine double bond cleaved (i.e., combination of i and ii above), with a rigid hydrophobic group which can mimic the effect of the lipid moiety of sugar conformation without allowing the lateral lipid packing that results in the formation of lamellar and micellar glycolipid structures in aqueous buffers.

Two such soluble $Gb_3$ analogs were produced using the oxidative hydrolysis procedure described above: an adamantyl and an acridine conjugate to the glycolipid acid. The adducts were filtered prior to use to ensure solubility. Unlike the free globotriaose, these species are potent inhibitors of $^{125}$I-VT1 binding to immobilized $Gb_3$ presented in a phospholipid matrix (Figure 9) (VT1 B subunit was used as positive control). These results demonstrate that these soluble membrane $Gb_3$ mimics are effective in preventing toxin binding to $Gb_3$ in a phospholipid bilayer within the micromolar range. This is the first description of any effective soluble competitive inhibitor of verotoxin receptor glycolipid binding. In this assay, the free globotriaose is totally ineffective.

The study shows that binding of bacterial pathogens to $Gg_3$ or $Gg_4$ is not inhibitable by the free galNAcb1-4gal dissacharide. This is thus analogous to the VT1/$Gb_3$ binding. In the case of the 'receptocides' described above, the antibiotic moiety has served to mimic the effect of the lipid component on the oligosaccharide receptor function. The generation of receptocides from soluble $Gg_3$ mimics, instead of $Gg_3$ itself, can also be achieved by combination of derivitizations e.g. where R= $\alpha$ adamantane in Figure 3.

## EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the following claims.

## CLAIMS

What is claimed is:

1. A method for treating a glycolipid mediated state in a subject comprising administering to a subject a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent, such that the glycolipid mediated state is treated.

2. The method of claim 1, wherein said glycolipid receptor moiety includes an oligosaccharide moiety coupled to a ceramide lipid base.

3. The method of claim 1, wherein said glycolipid receptor moiety is gangliotriaosyl ceramide galNAcβ1-4galβ1-4glc cer ($Gg_3$) or gangliotetraosyl ceramide galβ1-4galNAcβ1-4glc cer ($Gg_4$) and derivatives thereof.

4. The method of claim 1, wherein said active agent is an antibiotic.

5. The method of claim 4, wherein said antibiotic is a penicillin, cepham or a cephalosporin.

6. The method of claim 1, wherein said active agent is a carbocyclic compound.

7. The method of claim 6, wherein said carbocyclic compound is an adamantyl or an acridine derivative.

8. The method of claim 1, wherein said glycolipid mediated state is associated with a pathogenic microorganism.

9. The method of claim 8, wherein said pathogenic microorganism is a bacteria.

10. The method of claim 9, wherein said bacteria is selected from the group consisting of *Streptococcus pneumoniae, Streptococcus agalactiae (Gp. B.), Branhamella catarrhalis, Chlamydia trachomatis, Chlamydia pneumoniae, Clostridium perfringens, Clostridium difficile, Staphylococcus aureus, Klebsiella pneumoniae, Borrelia burgdorferi, Haemophilus influenzae, Haemophilus parainfluenzae,*

*Pseudomonas aeruginosa, Pseudomonas cepacia, Pseudomonas maltophilia, Neisseria gonorrhoeae, Neisseria meningitidis, Helicobacter pylori, Shigella dysenteriae, Shigella flexneri, Pasturella multocida, Coxiella burnetti, Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Salmonella typhymurium, Escherichia coli ATCC 6883, and Escherichia coli HB101/DH5a.*

11. The method of claim 9, wherein said bacteria is VTEC.

12. A method of modulating interaction between a pathogenic microorganism and a glycolipid in a subject comprising administering to a subject a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent, such that interaction between a pathogenic microorganism and a glycolipid is modulated.

13. The method of claim 12, wherein said glycolipid receptor moiety includes an oligosaccharide moiety coupled to a ceramide lipid base.

14. The method of claim 12, wherein said glycolipid receptor moiety is gangliotriaosyl ceramide galNAcβ1-4galβ1-4glc cer ($Gg_3$) or gangliotetraosyl ceramide galβ1-4galNAcβ1-4glc cer ($Gg_4$) and derivatives thereof.

15. The method of claim 12, wherein said active agent is an antibiotic.

16. The method of claim 15, wherein said antibiotic is a penicillin, cepham or a cephalosporin.

17. The method of claim 12, wherein said active agent is a carbocyclic compound.

18. The method of claim 17, wherein said carbocyclic compound is an adamantyl or an acridine derivative.

19. The method of claim 12, wherein said pathogenic microorganism is a bacteria.

20. The method of claim 19, wherein said bacteria is selected from the group consisting of *Streptococcus pneumoniae, Streptococcus agalactiae (Gp. B.), Branhamella catarrhalis, Chlamydia trachomatis, Chlamydia pneumoniae, Clostridium*

*perfringens, Clostridium difficile, Staphylococcus aureus, Klebsiella pneumoniae, Borrelia burgdorferi, Haemophilus influenzae, Haemophilus parainfluenzae, Pseudomonas aeruginosa, Pseudomonas cepacia, Pseudomonas maltophilia, Neisseria gonorrhoeae, Neisseria meningitidis, Helicobacter pylori, Shigella dysenteriae, Shigella flexneri, Pasturella multocida, Coxiella burnetti, Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Salmonella typhymurium, Escherichia coli ATCC 6883, and Escherichia coli HB101/DH5a.*

21. The method of claim 19, wherein said bacteria is VTEC.

22. A method treating a state characterized by the presence of a shiga-like toxin in a subject, comprising administering to a subject a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent, such that a state characterized by the presence of shiga-like toxin in the subject is treated.

23. The method of claim 22, wherein said shiga-like toxin is a SLTI, a SLTII, a SLTIII or any cytotoxin similar in both structure and function to Shiga toxin.

24. The method of claim 22, wherein said shiga-like toxin is verotoxin.

25. A compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent.

26. The compound of claim 25, wherein said glycolipid receptor moiety includes an oligosaccharide moiety coupled to a ceramide lipid base.

27. The compound of claim 25, wherein said glycolipid receptor moiety is gangliotriaosyl ceramide galNAcβ1-4galβ1-4glc cer ($Gg_3$) or gangliotetraosyl ceramide galβ1-4galNAcβ1-4glc cer ($Gg_4$) and derivatives thereof.

28. The compound of claim 25, wherein said active agent is an antibiotic.

29. The compound of claim 28, wherein said antibiotic is a penicillin, cepham or a cephalosporin.

30. The compound of claim 25, wherein said active agent is a carbocyclic compound.

31. The compound of claim 30, wherein said carbocyclic compound is an adamantyl or an acridine derivative.

32. A pharmaceutical composition comprising a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent and a pharmaceutically acceptable carrier.

33. The pharmaceutical composition of claim 32, wherein said active agent is an antibiotic.

34. A pharmaceutical composition for treating a glycolipid mediated state in a subject, comprising a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent and a pharmaceutically acceptable carrier, such that a glycolipid mediated state is treated.

35. The pharmaceutical composition of claim 34, wherein said active agent is an antibiotic.

36. A pharmaceutical composition for modulating interaction between a pathogenic microorganism and a glycolipid in a subject comprising a therapeutically effective amount of a therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent and a pharmaceutically acceptable carrier, such that interaction between a pathogenic microorganism and a glycolipid is modulated.

37. The pharmaceutical composition of claim 36, wherein said active agent is an antibiotic or a carbocyclic compound.

38. A packaged therapeutic composition for treating a glycolipid mediated state, comprising

a container holding a therapeutically effective amount of a therapeutic compound for treating a glycolipid mediated state in a subject, said therapeutic compound

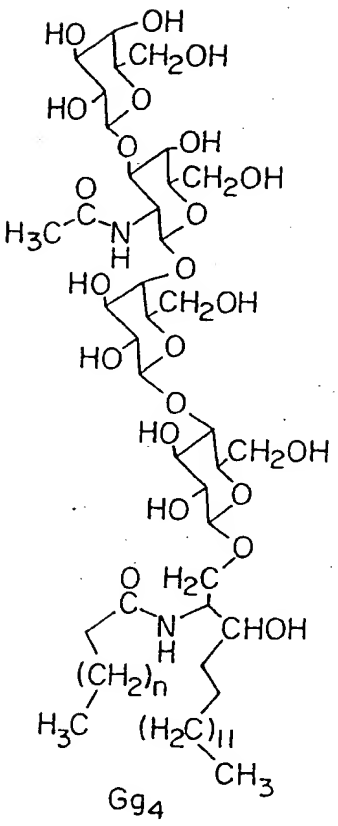
represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent; and

instructions for using said therapeutic composition for treating the glycolipid mediated state.

39. A packaged therapeutic composition for modulating interaction between a pathogenic microorganism and a glycolipid, comprising

a container holding a therapeutically effective amount of a therapeutic compound for modulating interaction between a pathogenic microorganism and a glycolipid in a subject, said therapeutic compound represented by the structure A-B, in which A is a glycolipid receptor moiety and B is an active agent; and

instructions for using said therapeutic composition for modulating interaction between the pathogenic microorganism and the glycolipid.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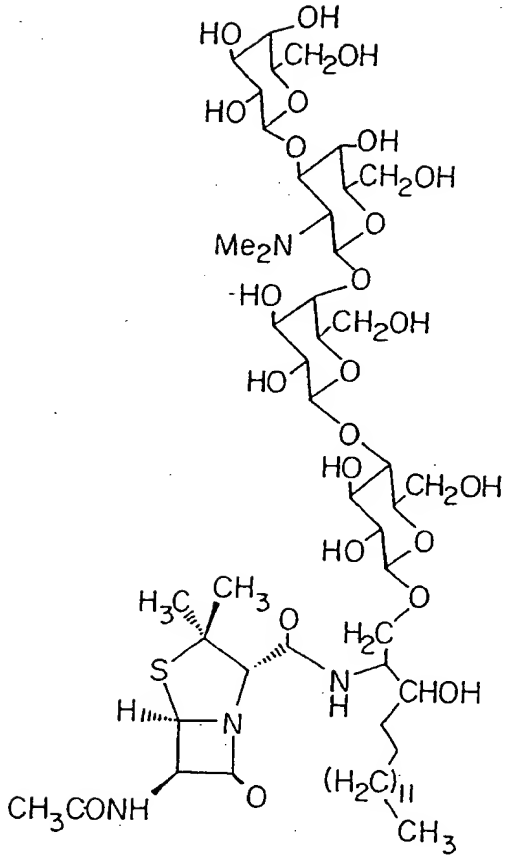
OH
CH2OH
HO
O
O
OH
CH2OH
H3C
C
O
N
H
O
HO
CH2OH
HO
O
O
HO
CH2OH
HO
O
O
O
H2C
C
O
N
H
CHOH
(CH2)n
H3C
(H2C)11
CH3

Gg4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CH2OH
HO
O
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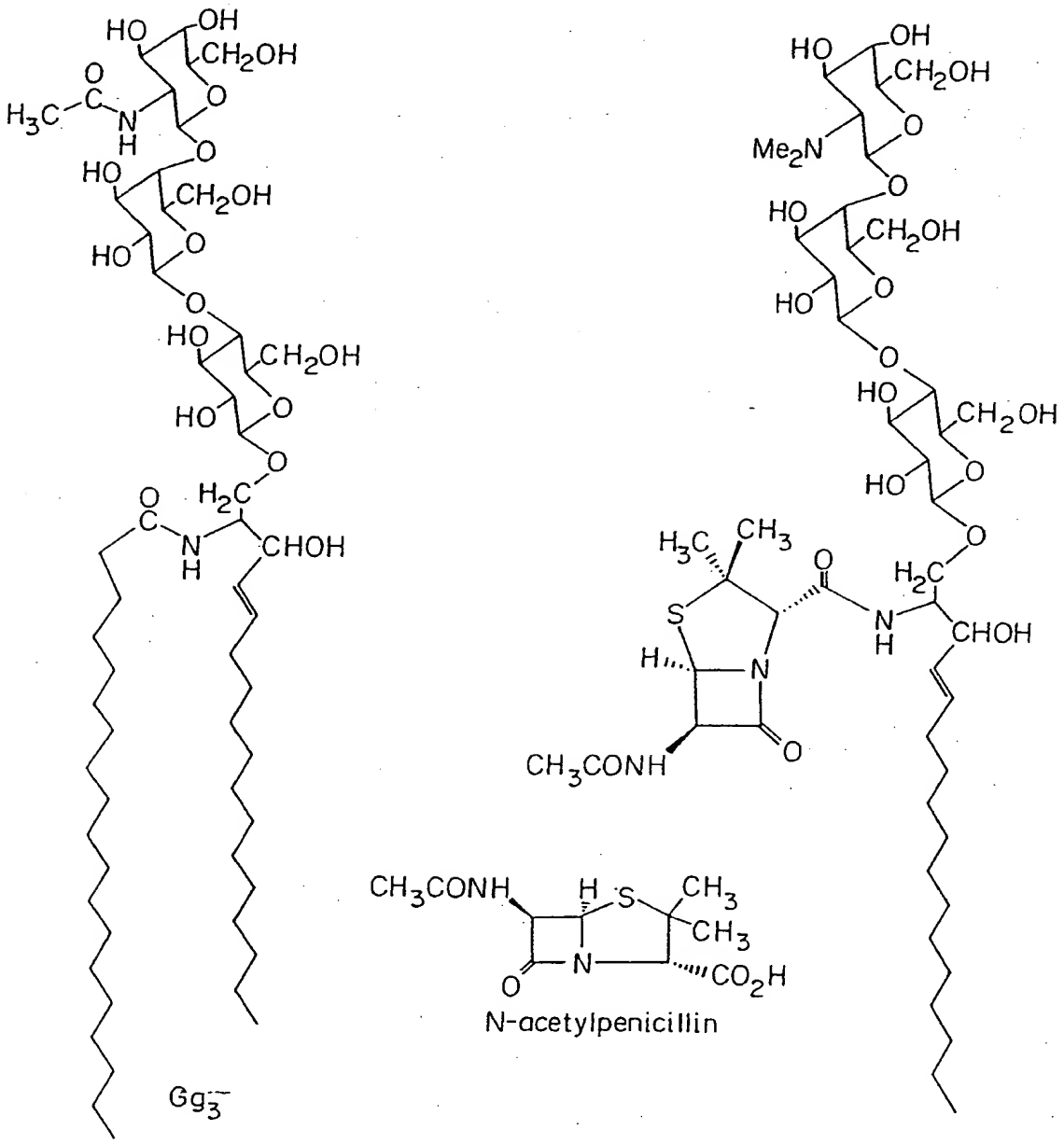
OH
CH2OH
Me2N
O
HO
CH2OH
HO
O
O
HO
CH2OH
HO
O
O
H3C
CH3
O
S
H
N
O
CH3CONH
H2C
N
H
CHOH
(H2C)11
CH3

FIG. 1A


HO
OH
CH2OH
O
H3C
C
N
H
O
HO
CH2OH
HO
O
HO
CH2OH
HO
O
O
H2C
C
O
N
H
CHOH
Gg3
Me2N
CH3
H3C
S
H
N
O
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CH3CONH
H
S
CH3
CH3
N
CO2H
O
N-acetylpenicillin

FIG. 1B

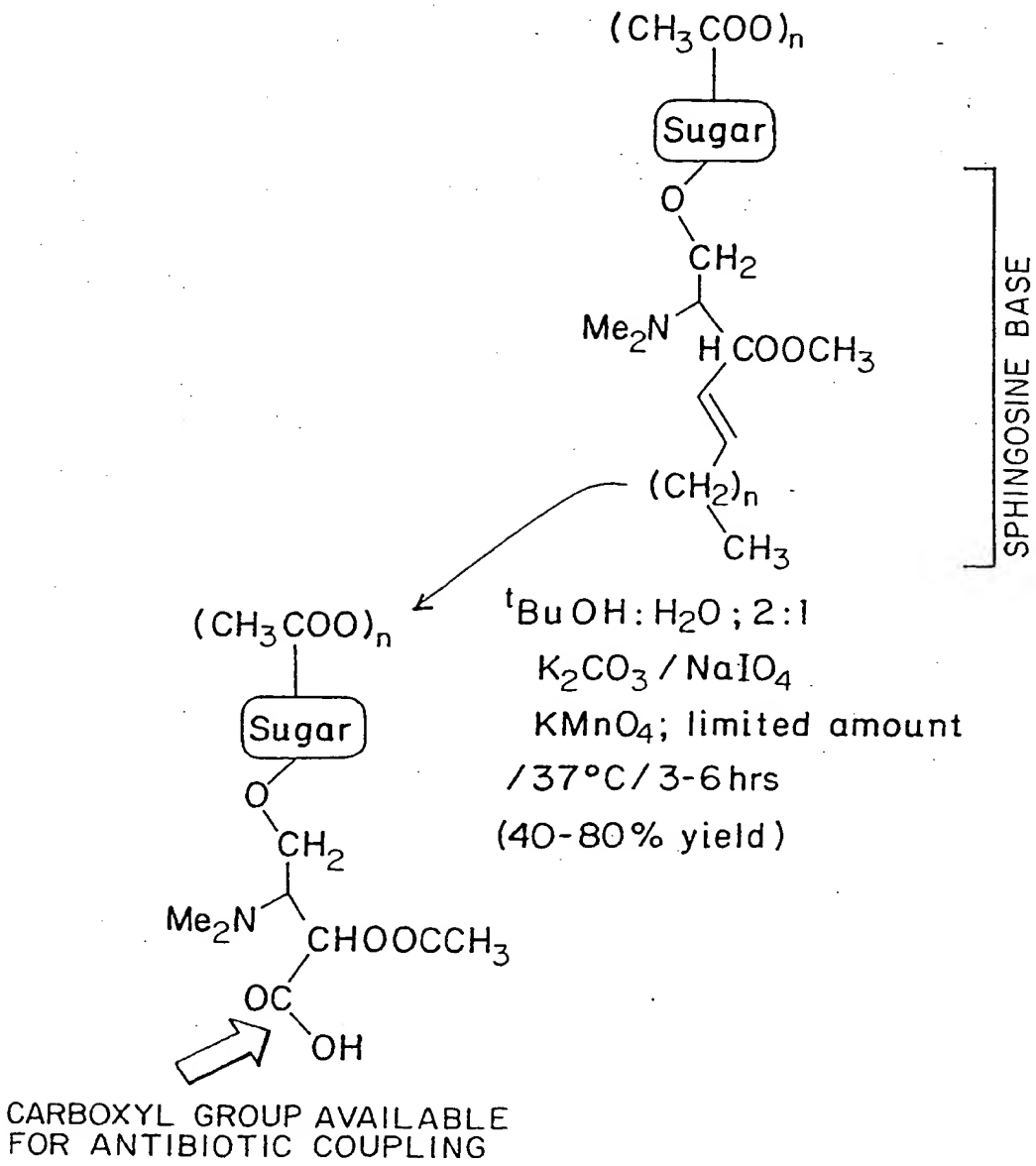
(CH3COO)n
Sugar
O
CH2
Me2N
H
COOCH3
(CH2)n
CH3
SPHINGOSINE BASE
tBuOH : H2O ; 2:1
K2CO3 / NaIO4
KMnO4; limited amount
/37°C/3-6hrs
(40-80% yield)
(CH3COO)n
Sugar
O
CH2
Me2N
CHOOCCH3
OC
OH
CARBOXYL GROUP AVAILABLE
FOR ANTIBIOTIC COUPLING

*FIG. 2A*

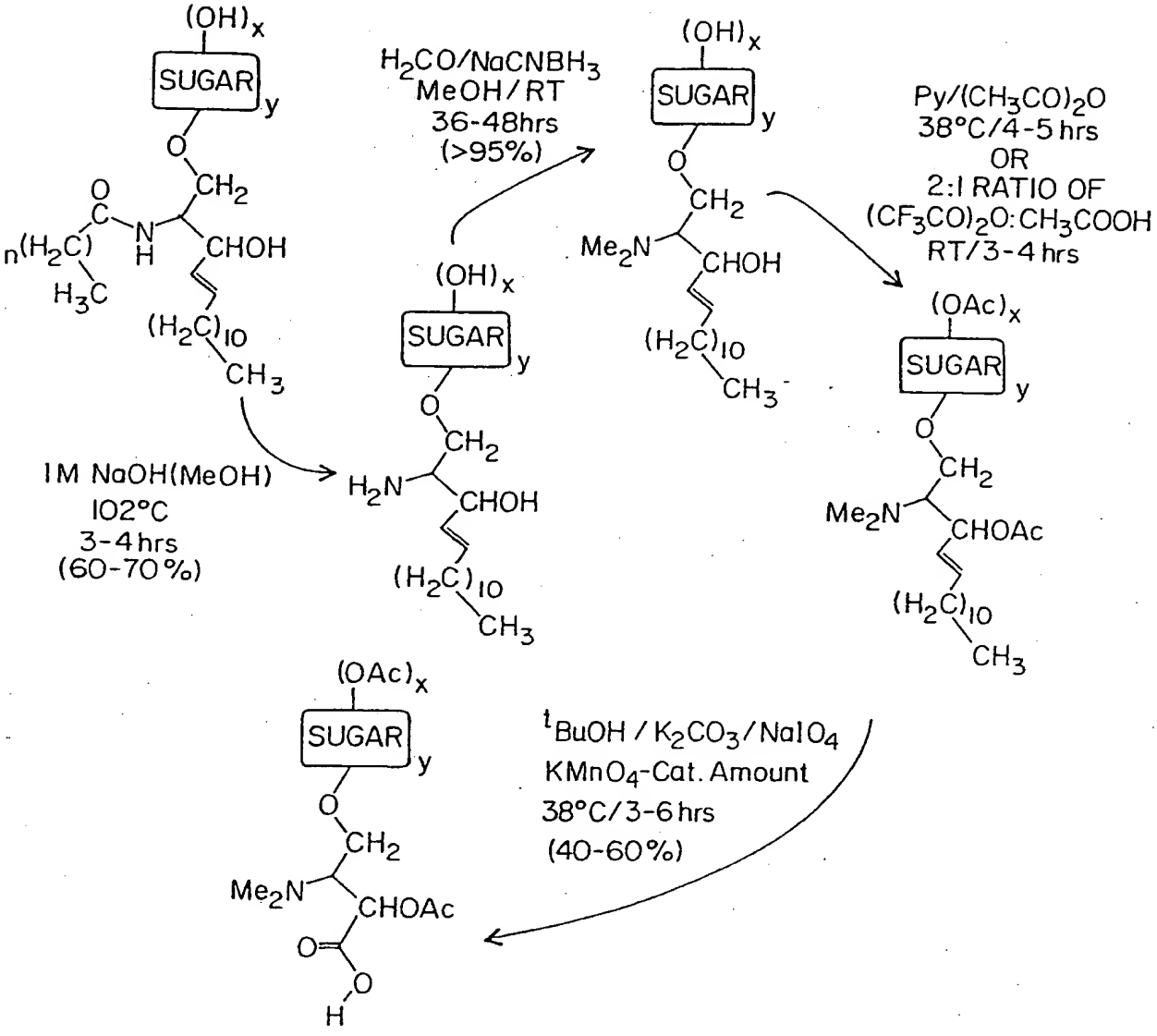
(OH)x
SUGAR
y
O
CH2
O
C
N
H
n(H2C)
H3C
CHOH
(H2C)10
CH3
H2CO/NaCNBH3
MeOH/RT
36-48hrs
(>95%)
Me2N
Py/(CH3CO)2O
38°C/4-5 hrs
OR
2:1 RATIO OF
(CF3CO)2O:CH3COOH
RT/3-4 hrs
(OAc)x
CHOAc
1M NaOH(MeOH)
102°C
3-4hrs
(60-70%)
H2N
tBuOH / K2CO3/NaIO4
KMnO4-Cat. Amount
38°C/3-6 hrs
(40-60%)

FIG. 2B

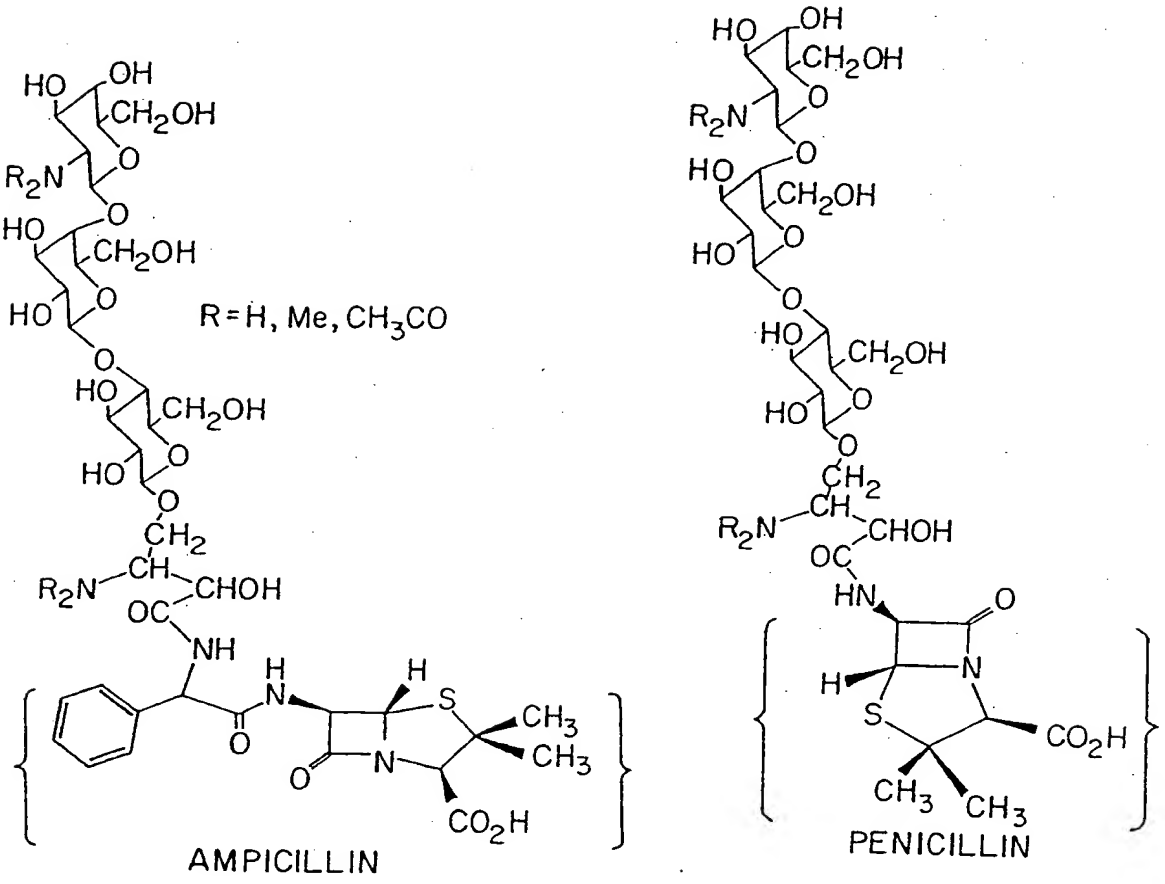


FIG. 3

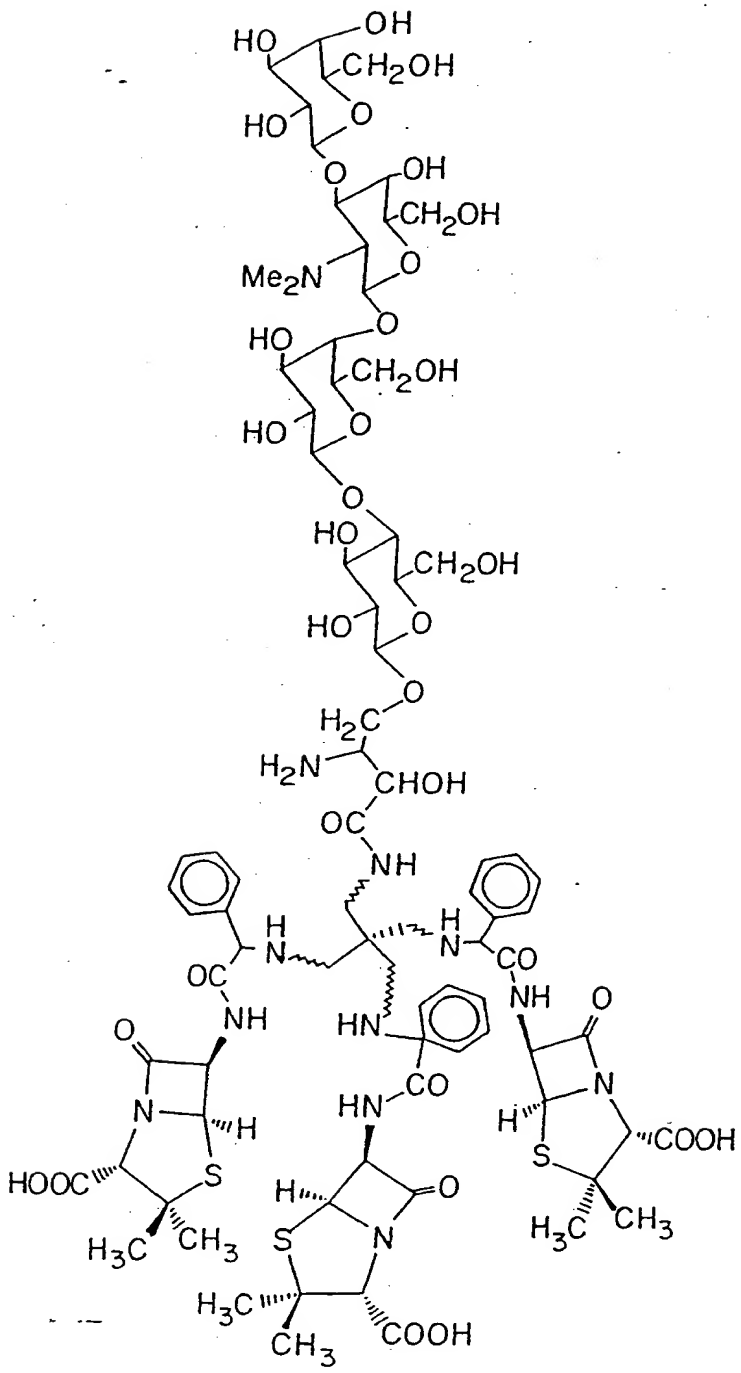

FIG. 4

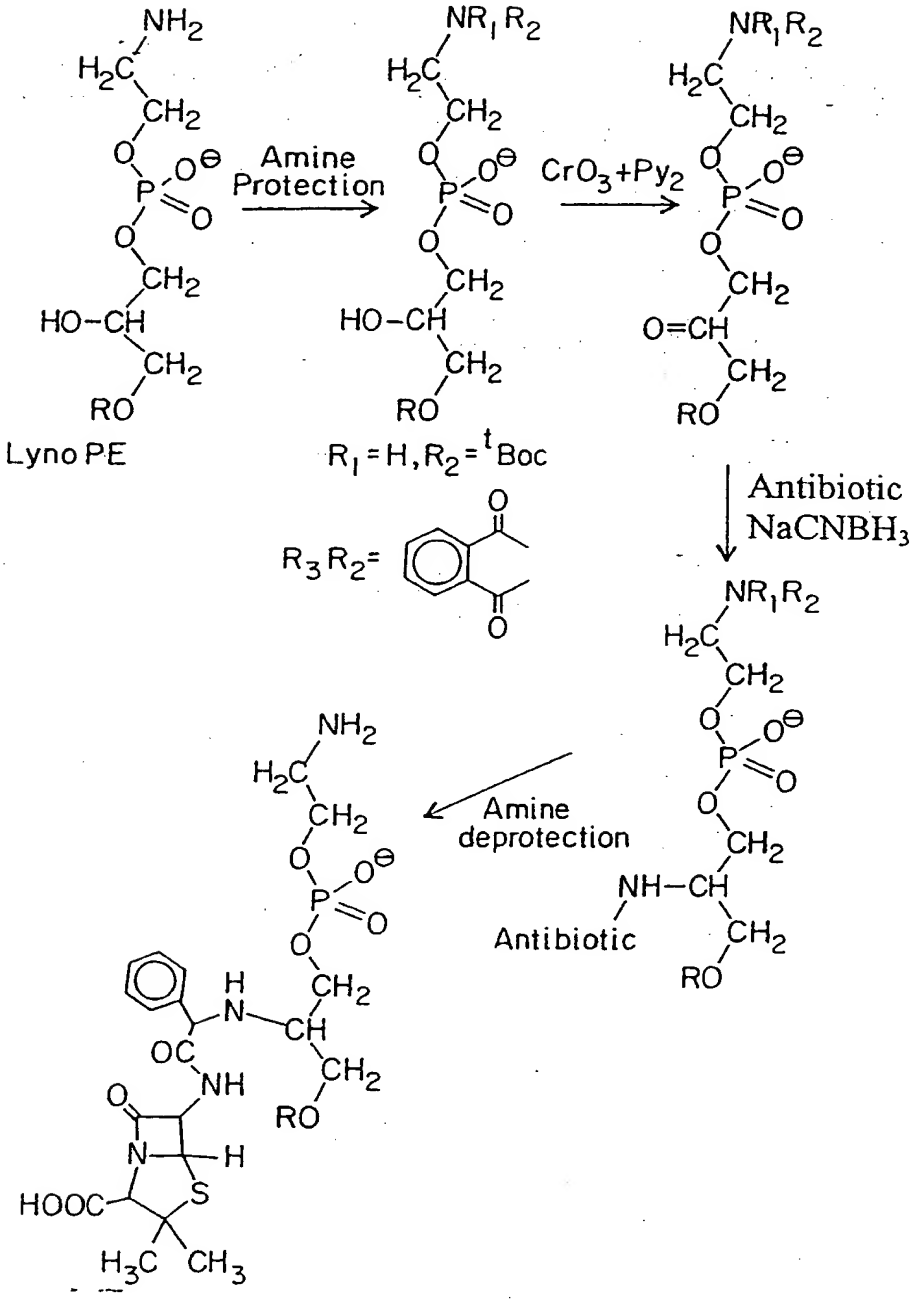
Amine Protection
$CrO_3+Py_2$
Lyno PE
$R_1=H, R_2={}^tBoc$
$R_3R_2=$
Antibiotic
$NaCNBH_3$
Amine deprotection
Antibiotic

*FIG. 5*


FIG. 6

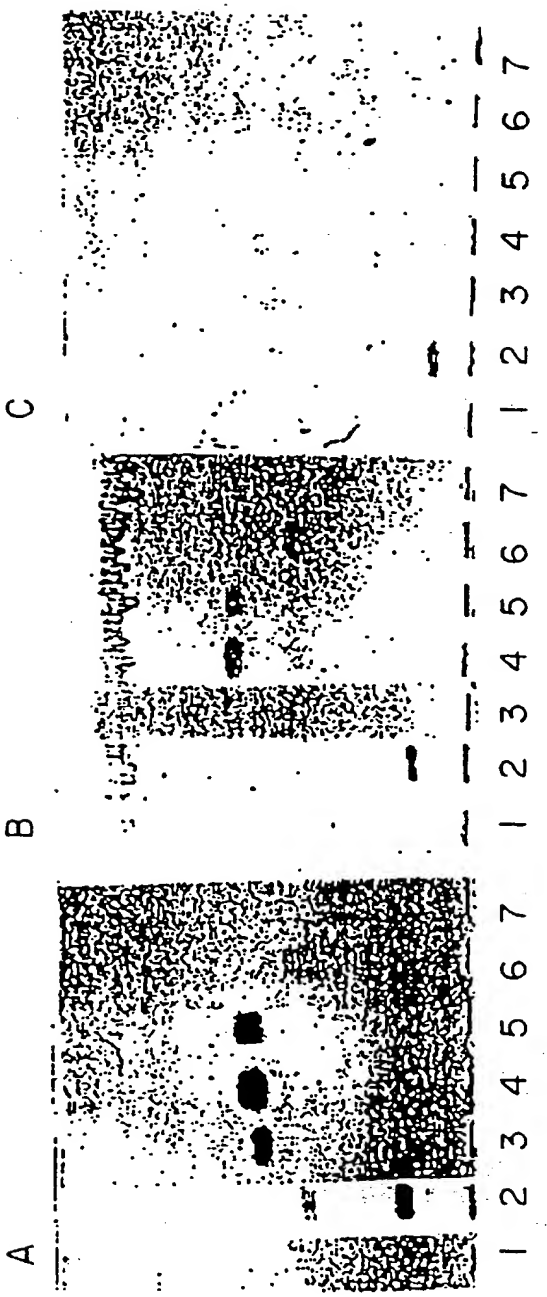


FIG. 7

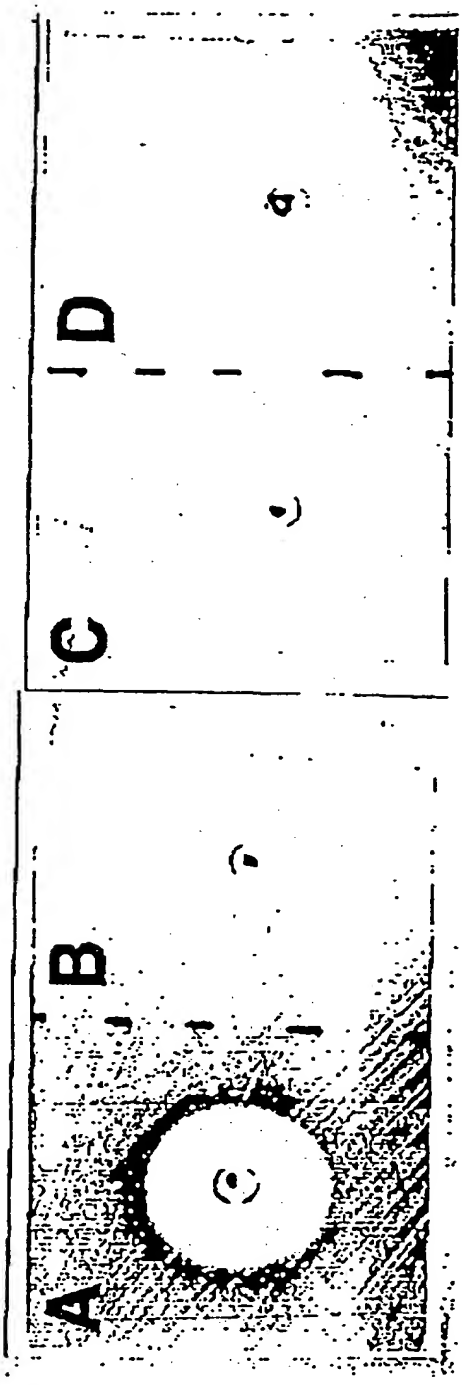


FIG. 8

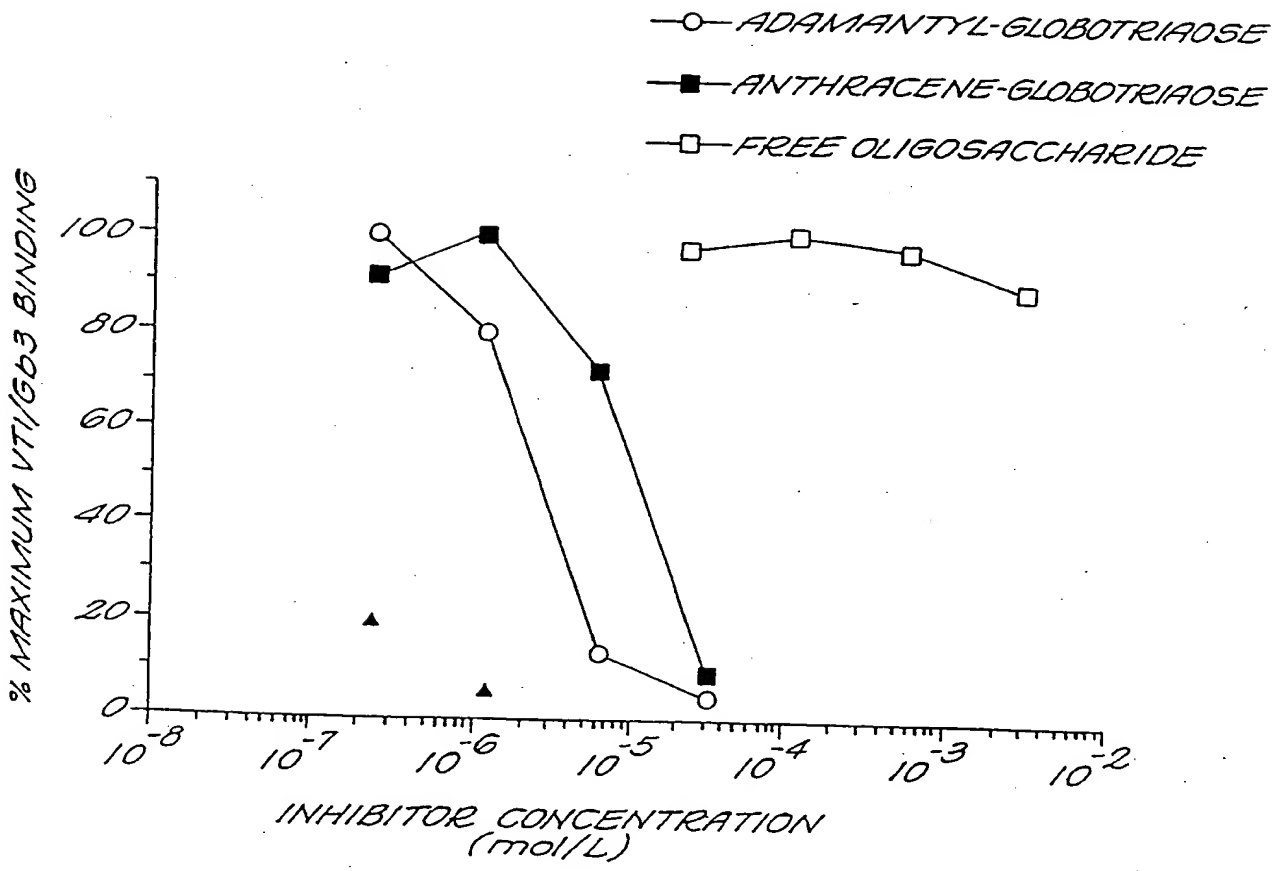
ADAMANTYL-GLOBOTRIAOSE
ANTHRACENE-GLOBOTRIAOSE
FREE OLIGOSACCHARIDE
% MAXIMUM VTI/603 BINDING
100
80
60
40
20
0
$10^{-8}$
$10^{-7}$
$10^{-6}$
$10^{-5}$
$10^{-4}$
$10^{-3}$
$10^{-2}$
INHIBITOR CONCENTRATION (mol/L)

FIG. 9

# INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/CA 98/00142

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | US 5 466 681 A (KRIVAN HOWARD C ET AL) 14 November 1995<br>see column 3, line 1-10; figures 1,2<br>see column 4, line 9-21 - line 54-67;<br>claims 1-3,5-7,9,10; examples 1-4 | 1-39 |
| X,P | US 5 696 000 A (KRIVAN HOWARD C ET AL) 9 December 1997<br>see column 11, line 22-55; tables 1,3<br>& WO 92 02817 A | 1-39 |
| X | US 5 463 092 A (HOSTETLER KARL Y ET AL) 31 October 1995<br>see abstract; claims 1,11 | 1-4,8, 12,13, 15,19, 25,26, 28,32, 33,36-39 |

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 July 1998 | 12/08/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Gonzalez Ramon, N |

Form PCT/ISA/210 (second sheet) (July 1992)

# INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/CA 98/00142

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ' | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X,P | WO 97 18790 A (UNIV MONTANA RES DEV INST ;PASCUAL DAVID (US); BOND CLIFFORD (US);) 29 May 1997<br>see page 27, line 4-28; claims 1-4,9,23,26,27; examples 8,14,16<br>see page 61; claims 38,39,41,42,44,46,47<br>see page 71 - page 72.<br>--- | 1-4,8, 12,13, 15,19, 25,26, 28,32, 33,36-39 |
| Y | WO 92 11015 A (MICROCARB INC ;HSC RESEARCH AND DEV (CA)) 9 July 1992<br>see abstract; claims 1-3; examples 1,3<br>see page 8, line 10-20<br>--- | 1-39 |
| A | WO 89 11272 A (LIPOSOME CO INC) 30 November 1989<br>see abstract; claims 7,9,11,12; examples 9-11<br>--- | 1-39 |
| Y | US 4 464 360 A (LEFFLER HAKON ET AL) 7 August 1984<br>see abstract; claim 1; table 2<br>--- | 1-39 |
| Y | ARAB S ET AL: "Expression of the verotoxin receptor glycolipid, globotriaosylceramide, in ovarian hyperplasias."<br>ONCOL RES, 1997, 9 (10) P553-63, UNITED STATES, XP002071680<br>see page 561 - page 562<br>--- | 1-39 |
| A | LINGWOOD C A: "Aglycone modulation of glycolipid receptor function"<br>GLYCOCONJUGATE JOURNAL, 13 (4). 1996. 495-503., XP002071690<br>see abstract; figure 1<br>see page 498, column 1, paragraph 2 - column 2, paragraph 3<br>see page 501<br>--- | 1-39 |
| Y | BITZAN MM ET AL: "Inhibition of Helicobacter pylori and Helicobacter mustelae binding to lipid receptors by bovine colostrum."<br>J INFECT DIS, APR 1998, 177 (4) P955-61, UNITED STATES, XP002071679<br>see abstract<br>see page 957, column 2 - page 958, column 1<br>see page 959, column 2, paragraph 3 - page 960, column 1<br>----- | 1-39 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 98/ 00142

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-24
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-24
is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1,2,6,8,12,22,25,32,
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
34,36-39

See FURTHER INFORMATION Sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1 [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/00142

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
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| | | WO 9202817 A | 20-02-1992 |
| US 5463092 A | 31-10-1995 | US 5194654 A | 16-03-1993 |
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| WO 9718790 A | 29-05-1997 | AU 1122697 A | 11-06-1997 |
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| | | DK 563256 T | 28-08-1995 |
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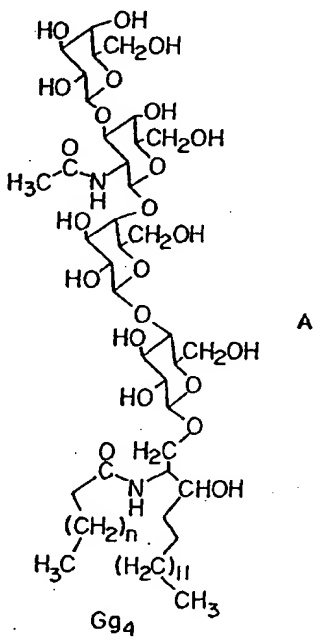
(54) Title: ANTIBIOTIC–LIGAND CONJUGATES AND METHODS OF USE THEREOF

(57) Abstract

Methods for treating a glycolipid mediated state in a subject are described. An effective amount of at least one therapeutic compound represented by the structure A–B, in which A is a glycolipid receptor moiety and B is an active agent, is administered to a subject, such that treatment of the glycolipid mediated state occurs. Methods also include administering and effective amount of at least one therapeutic compound, or a pharmaceutically acceptable salt thereof, to a subject such that a disease state associated with a SLT is treated. Packaged pharmaceutical compositions for treating SLTs are described. The package includes a container for holding an effective amount of a pharmaceutical composition and instructions for using the pharmaceutical composition for treatment of SLT. The pharmaceutical composition includes at least one therapeutic compound for modulating a SLT in a subject.



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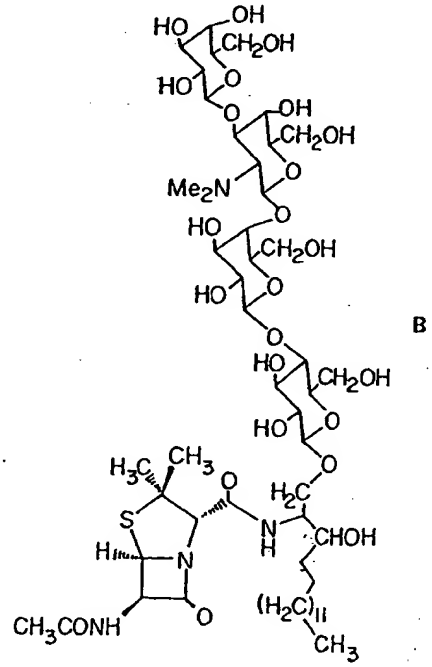

B

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00142

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 20 July 1998 | 12/08/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Gonzalez Ramon, N |

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00142

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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# INTERNATIONAL SEARCH REPORT

.ernational application No.
PCT/CA 98/00142

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-24
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 1-24 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1,2,6,8,12,22,25,32,
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
34,36-39

See FURTHER INFORMATION Sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No. PCT/CA 98/00142

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1,2,6,8,12,22,25,32,34,36-39

In view of the large number of compounds, which are defined by the general definition in the independent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application. (see Guidelines, Chapter III, paragraph 2.3).

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Information on patent family members

International Application No
PCT/CA 98/00142

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